GENETICS

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A NOTE ON SOME TECHNIQUES OF OBTAINING GENETIC SEGREGATION IN APOMICTIC STRAINS OF THE GUAYULE RUBBER PLANT

REED C. ROLLINS AND D. G. CATCHESIDE
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Received October 24, 1950

UP to the present, the most successful strains of guayule (*Parthenium argentatum*) in cultivation for rubber production have been facultatively apomictic. That is, plants of these strains produce seeds without the usual fertilization process taking place. The chromosome number is maintained the same from parent to offspring because the usual reduction of chromosomes preceding egg-cell formation does not take place. Thus, when apomixis is strictly operative in these plants, the egg-cell is never fertilized by a sperm nucleus, but the chromosome number remains unaltered from generation to generation. Under this arrangement, no opportunity is afforded for the genetic differentiation that ordinarily occurs in plants when seed is produced. The offspring are genetically and phenotypically like the mother plant.

The genetic stability, over an indefinite number of generations of a given apomictic strain, has certain advantages. In general, its purity is assured and simply maintained. To have the ultimately superior strain of guayule also apomictic is a goal worthy of considerable effort. The problem is to develop this superior strain, without at the same time losing apomixis, for the usual breeding methods are based on the use of the antithetic sexual process. The problem then is to release the genetic potential of the apomictic strains without losing apomixis by the procedures followed.

Certain known features of guayule open several possibilities of bringing about genetic segregation in the apomictic types. Since both sexual and apomictic strains occur, the most obvious method is to cross these using the sexual strains to receive the pollen. So far as is known, meiosis occurs quite normally preceding pollen production in the apomictic plants, that is as normally as in most polyploid plants, so that full genetic segregation is achieved. However, where apomictic and sexual types have been crossed, the first and second generation progeny plants are mostly sexual. Definitive results are not available to show the ultimate possibilities of reestablishing apomixis, but under the most favorable circumstances it is certain to require a long breeding procedure. Another difficulty, encountered where the above method is used, stems from the tendency toward non-reduction of chromosomes at megasporogenesis in the apomictic plants. Some genes involved in non-reduction are inevitably brought into the resulting progenies and even though we do not know how they operate specifically, they do complicate the reproductive process. Triploids become more frequent and incompatibilities appear to be increased.

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Other ways in which genetic segregation and recombination could be achieved among apomictic plants rest on the fact that apomixis in guayule is not obligate. Apomictic strains differ in the amount of apomixis they show, varying from around 80 to over 95 percent. By crossing apomictic strains and utilizing the few percent of sexually produced offspring some progress might be made. One difficulty is that of recognizing these plants and of finding the rare improved one without an inordinate amount of work. Moreover, there are a number of complicating factors, which can best be understood if the salient features of the divergent types of embryo production (ESAU 1946; POWERS and ROLLINS 1945) are summarized. In 72 chromosome apomictic strains of guayule a majority of the functional embryo sacs are formed without the megaspore mother cell having undergone reduction in chromosome number in the production of the megaspore. While the course of development in guayule shows that most unreduced megaspores are produced without any meiotic process, it appears that a few are produced following one meiotic division, the first. Any crossing over in these would give the opportunity of at least a limited amount of recombination (ROLLINS 1945). In a minority reduction does occur, so that although the majority of the functional eggs are unreduced a minority are reduced and presumably genetic segregation will have occurred in their formation. Most eggs commence to produce an embryo, irrespective of whether pollination and the opportunity of fertilization has occurred. However, no apomictic embryos continue development unless fertilization of the primary endosperm nucleus occurs. While fertilization is necessary to initiate endosperm formation and thus support continued embryo development, fertilization of the egg is unnecessary and is indeed precluded in the majority of the embryo sacs by the precocious initiation of embryo development. As a result of non-fertilization of some of the reduced eggs, "haploid"[1] individuals arise. These are relatively uncommon presumably because the "haploids" have a rather low viability.

The eggs may be fertilized in a minority of the embryo sacs and so form amphimict embryos. However, the majority of such embryos, whether from selfing or from a cross between apomict strains are "triploid"[1] with respect to their immediate parents. To the unreduced chromosome number found in the egg is added the reduced number from the pollen. Such "triploids" are most often aberrant and unpromising. There is usually also a small percentage of the progeny of the cross that arises by fertilization of reduced eggs by sperms from reduced pollen grains. These plants offer some promise and may be utilized in a long range breeding program.

In sum, then, the progeny of a 72 chromosome apomictic guayule is generated in six ways giving four different types of progeny. Unreduced eggs formed purely vegetatively if unfertilized give rise to maternal 72 chromosome plants; if fertilized they give rise to "triploids" which are recognizable

[1] The words "haploid" and "triploid" are used in quotation marks because while these plants are haploid or triploid with respect to their immediate parents, they are not haploid or triploid for the species, but rather diploid and hexaploid.

by morphological features. Unreduced eggs formed by ameiosis, in which the second meiotic division has been omitted, if unfertilized give rise to " deviating maternal " 72 chromosome plants; if fertilized they give rise to " triploids." Reduced eggs if unfertilized give rise to " haploid " 36 chromosome plants; if fertilized they give rise to genetically different 72 chromosome plants. These 72 chromosome plants are the raw material for a selection program. At present, their potentialities are unknown and very little is known as to what the ultimate effects would be on apomixis and non-reduction if they were selected. Moreover, such plants are not only relatively rare in a given progeny, but are generally recognizable only if morphologically different from the maternal type and so are difficult to discover.

A novel technique of obtaining numerous genetically different 72 chromosome plants, and so of obtaining segregation in apomictic strains with the possibility of not losing their apomictic properties, appears promising. This technique utilizes the 36 chromosome " haploid " plants given by certain 72 chromosome apomictic strains. These plants are recognizable by their smaller size, in all respects, though by no means all small narrow leaved aberrant plants in a progeny are " haploid." In these " haploids," it appears that all the functional eggs are unreduced. Chromosome behavior in megasporogenesis has not been studied, but in the pollen mother cells of the " haploids " eighteen bivalents are formed very regularly and reduction is the rule, but the pollen is infertile. The chiasma frequency is slightly lower than in 36 chromosome sexual guayule, namely 19.75 per cell, as compared with 21.44 per cell in the sexual plants. When a " haploid " was used as a female parent and pollinated by pollen from the original 72 chromosome parent, two types of plants were found in the progeny. Those of one kind were all like the maternal " haploid " in appearance and chromosome number, those of the other kind were vigorous and had 72 chromosomes. Evidently both arise from unreduced eggs, the one from unfertilized eggs and the other from fertilized eggs. In the latter a reduced 36 chromosome number from the 72 chromosome pollen parent was added to the unreduced 36 chromosome number present in the egg cell. Each plant thus produced is likely to differ from the original apomictic strain. They are to be expected to show diversity in respect of genetic segregation that has occurred in the pollen parent. Further, different " haploids " may be expected to show different combining properties with the same pollen parent. Some " haploids " may be expected to be relatively elite in that with any pollen parent they give more desirable progeny than do other " haploids." For selection purposes, the progenies may be tested for the various desirable qualities, including high rubber content, low resin and high apomixis.

A comparison of rubber analyses of the small population available for testing is very interesting as table 1 shows. The 72 chromosome " hybrid " plants are considerably better in rubber content than the " haploid " sister plants and are also much better than 72 chromosome plants of variety 593 used as a standard of comparison. The plants sampled for the data of table 1 were

all planted at the same time and were grown in the field under approximately the same conditions. However, no attempt was made to control rigorously the environmental factors related to the growth of the plants.

The data, which are unfortunately not very extensive, have been subjected to a *t* test, the results of which are summarized in table 2. It is quite clear that the rubber content of the "hybrids" is significantly higher than either the "haploids" or the variety 593 plants, which are not significantly different from one another. The resin contents of all are, on the other hand, not sig-

TABLE 1

Rubber and resin[2] data on guayule planted in experimental plots at Stanford University, April, 1945.

Plant number	Origin	Somatic chromosome number	Date sampled	Rubber percent	Resin percent
Maternal "haploids"					
45–34–2	6879–17 open pollinated	36	Feb. 1949	5.45	5.84
45–34–4	6879–17 open pollinated	36	" "	6.37	5.32
45–38–4	6879–17 × 42354-II	36	" "	4.62	5.43
45–38–5	" × "	36	" "	6.28	6.80
			Means	5.68 ± 0.409	5.85 ± 0.337
"Hybrids" from "haploids"					
45–38–1	6879–17 × 42354-II	72	Feb. 1949	9.03	6.50
45–38–3	" × "	72	" "	6.39	6.83
45–39–1	" × "	72	Feb. 1947	10.48	4.40
45–39–2	" × "	72	Feb. 1949	9.45	7.49
			Means	8.84 ± 0.822	6.31 ± 0.668
Variety 593 maternals					
45–15–4	593-V × 43691-IV	72	Feb. 1949	6.12	5.98
45–15–4	" × "	72	Feb. 1947	6.40	5.62
45–15–5	" × "	72	" "	7.57	6.26
45–15–11	" × "	72	" "	5.33	5.10
			Means	6.36 ± 0.464	5.74 ± 0.250

[2] Analyses were provided from the Rubber Laboratory, Guayule Research Project, Division of Rubber Plant Investigations, United States Department of Agriculture, Salinas, California.

nificantly different from one another. The rubber and resin contents of the "hybrids," derived from the "haploids," appear to be more variable than those of either of the other groups of plants, suggesting a diversity in genotype arising from segregation in the plants used as pollen parents. Amongst the "hybrid" group the rubber content of plant 45–38–3 and the resin content of plant 45–39–1 are significantly lower at the 6 or 7 percent level than the respective means of the group.

Considering all the plants as a single group, the mean rubber content percent is 6.958 ± 0.521 and the mean resin content percent is 5.964 ± 0.249.

Amongst the " hybrids " arising from the " haploids," plants 45–38–1, 45–39–1 and 45–39–2 have significantly higher rubber contents. Plant 45–39–1 has in addition a significantly lower resin content making it even more desirable, while plant 45–39–2 has a significantly higher resin content which reduces its value.

The data, even though meagre, are worth recording for the benefit of others who may be able to follow them up, since neither of the authors will be able to do so. The population sampled was not large enough to estimate the amount of segregation achieved. Indeed, the original cross of the " haploid," back to the plant from which it arose, was not designed to bring out this point. The incidental sampling of one " hybrid " (No. 45–39–1) in 1947, showed such a high rubber content that we were lead to sample the rest of the population for comparison. Why the rubber content showed such a remarkable increase in three of the " hybrids " while the resin content showed much smaller changes, is not understandable from the data we have. It does not appear as though there is any particular correlation between rubber and resin contents. The results suggest that high rubber content tends to be recessive to low content. These conclusions are consistent with the results obtained from interspecific crosses between guayule and *Parthenium stramonium* where the resin contents of 54 chromosome and 90 chromosome F_1 hybrids were 5.62 ± 0.43 and 6.35 ± 0.29 percent respectively, whereas the rubber contents were 2.18 ± 0.39 and 4.61 ± 0.32 percent for the same groups of plants (ROLLINS, CATCHESIDE and GERSTEL 1947).

TABLE 2

t test of rubber and resin data on guayule recorded in Table 1.

Groups of plants compared	Rubber content Differences of means	*t*	P	Resin content Differences of means	*t*	P
"Haploids" with "Hybrids"	3.16 ± 0.92	3.44	0.01–0.02	0.46 ± 0.75	0.62	0.5–0.7
"Hybrids" with Maternals	2.48 ± 0.94	2.63	0.02–0.05	0.57 ± 0.72	0.80	0.3–0.5
"Haploids" with Maternals	0.68 ± 0.62	1.10	0.3 –0.5	0.11 ± 0.42	0.26	0.8–0.9

One cannot but be impressed by the great increase in rubber content obtained by the latter method and further to speculate as to what results might be obtained if the " haploids " were crossed to unrelated 72 chromosome strains rather than to their own parent. It is also possible that particularly elite " haploids " could be selected capable of yielding, after outcrossing, apomicts combining many good qualities.

SUMMARY

Since the most successful strains of guayule, from an agricultural point of view, are largely apomictic in their seed production, the problem of releasing their genetic potential for breeding purposes without losing the apomictic

feature is a pertinent one. A method is described whereby 36 chromosome "haploid" plants given by certain 72 chromosome apomictic strains are utilized to achieve this objective. Results from rubber and resin analyses of a small progeny of 72 chromosome "hybrid" plants produced from 36 chromosome "haploids," together with comparative materials, showed that genetic segregation did occur and that such a method may possibly be used to increase the rubber content of apomictic strains of guayule.

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THE INCOMPATIBILITY FACTORS AND A RELATED GENE IN SCHIZOPHYLLUM COMMUNE[1]

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THE genetic basis of incompatibility in the higher fungi was described for the first time by KNIEP (1920) using the tetrapolar hymenomycete *Schizophyllum commune*. Subsequent work, in various countries, fully confirmed KNIEP's statement and extended its validity to numerous other basidiomycetes. This later work, however, though voluminous, added little fundamental new knowledge. It is only very recently, moreover, that the data obtained have been adequately analyzed and the deductions that can be drawn clearly summarized (WHITEHOUSE 1949a).

The prevalence of multiple-allele heterothallism among plants as opposed to dioecious sex among animals is of interest and may be connected with the directional motility of animals which would tend to reduce the loss of overall fertility inherent in dioecious sex. Among plants multiple-allele heterothallism is widespread among two successful diversified groups: the angiosperms, and the basidiomycetes. WHITEHOUSE (1950a) has suggested, on extensive grounds, that the major factor in the evolution of the angiosperms was the appearance of multiple-allele heterothallism. He concludes that the character has arisen very seldom in evolutionary history.

Whatever may be the origins of multiple-allele heterothallism, a gene with such a large number of equivalent alternate states as that controlling incompatibility presents many interesting problems the solution of which will throw light on some of the fundamental aspects of physiological and evolutionary genetics.

Compatibility in the tetrapolar hymenomycetes is controlled by series of multiple alleles at two unlinked loci. When two monokaryotic (haploid) mycelia having different alleles at both of these loci, meet, there is a mutual exchange of nuclei and both mycelia are dikaryotized. Dikaryotic mycelia produce fruiting-bodies; nuclear fusion occurs in the young basidium, followed by reduction and the formation of four basidiospores. All fruiting-bodies are thus heterozygous at both loci and give rise to monokaryons of four different mating types. Pairs of monokaryons with the same alleles at one or at both loci are sterile but in *S. commune* it has recently been shown (PAPAZIAN 1949, 1950c) that where the alleles are the same at only one locus characteristic heterokaryons are formed. These heterokaryons have recognizable morphologies and they dikaryotize unilaterally; they are capable of dikaryotizing monokaryons of appropriate mating type but are never themselves di-

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karyotized. Heterokaryons in which the *A* factors are the same have been named Flat (F), and those with common *B* factors, Barrage (B). The various reactions which would be obtained by confronting the four incompatibility types from a single fruiting-body among themselves are shown in table 1.

TABLE 1

Mating reactions in S. commune.

	A^1B^1	A^1B^2	A^2B^1	A^2B^2
A^1B^1	–	F	B	+
A^1B^2	F	–	+	B
A^2B^1	B	+	–	F
A^2B^2	+	B	F	–

+ = Dikaryon formed
– = Overlap
F = Flat (non-fertile heterokaryon)
B = Barrage (non-fertile heterokaryon)

An aberrant type of mycelium, Streak (*s*), which will be studied from a genetical point of view in the present paper has been described in detail in a previous publication (PAPAZIAN 1950c). The only items which need be repeated here are that Streak is a monokaryon and dikaryotizes unilaterally other monokaryons of compatible mating type. One of the most interesting features of Streak mycelia is their origin. Streak mycelia have never been found to arise spontaneously from wild type monokaryons but they can be very readily obtained from Flat cultures, especially after blending in a Waring Blendor (PAPAZIAN 1950c).

Throughout the following account an unavoidable verbal inconsistency must be admitted. For the sake of clarity the *A* and *B* incompatibility factors are treated as corresponding to single genes or single loci, in spite of the fact that the conclusion is drawn that the *A* factor corresponds to at least two distinct genes.

MATERIALS AND METHODS

The cultures used in the present work were the same as those used in a previous publication (PAPAZIAN 1950c). The nomenclature is identical to that used previously. Mutant alleles, or possible mutant alleles, at the incompatibility loci are symbolized by *a* and *b* corresponding to the *A* and *B* loci. They are distinguished among themselves by superscripts.

Of three essentially new techniques that were used, two, concerned with the isolation of tetrads and the production of fruiting-bodies from dikaryons, have been described elsewhere (PAPAZIAN 1950a, 1950b). The third new technique concerns the analysis of monokaryons obtained from a fruiting-body whose genotype, as regards the incompatibility alleles, is known. The number of matings required in this technique is never more than half that required by other techniques in common usage and the work involved is thus greatly reduced. The monokaryons to be tested are each mated with two testers of appropriate mating type. Thus, if the fruiting-body had the constitution $A^1A^2B^1B^2$, the spores from it would fall into the four classes A^1B^1, A^2B^2,

A^1B^2, A^2B^1, and appropriate testers would be A^1B^3 and A^3B^1. The reactions of monokaryons of the various incompatibility types with these two testers would be:

	A^1B^3	A^3B^1
A^1B^1	F	B
A^2B^2	+	+
A^1B^2	F	+
A^2B^1	+	B,

This method suffers from the disadvantage that any mutants or abnormal behavior will not, in most cases, be recognized.

CROSSES NOT INVOLVING STREAK

The spores from three wild fruiting-bodies, XV(ad), XXV(mad), and I(arg) (collected from the Adirondack mountains, N. Y., Madison, Wisc., and the Argonne Forest Preserve, Ill., respectively), and from various fruiting-bodies grown in the laboratory from crosses between these stocks, were analyzed for mating type. These analyses were made primarily to obtain monokaryons of various mating types required for other experiments. The results of these analyses are shown in table 2. Except for the last item, no particular significance is attached to these results. They are recorded as evidence that no lethals or other factors that would seriously affect the results of further work, were present in the stocks; and as data to be compared, and added to, similar results of other workers. The last item in table 2 (fruiting-

TABLE 2

Distribution of mating types in wild and cultured fruiting-bodies.

Fruiting body	Origin of fruit.	Number	Mating types							
XV(ad)	Wild	8	1	A^3B^3	2	A^4B^4	4	A^3B^4	1	A^4B^3
XXV(mad)	Wild	10	3	A^3B^1	3	A^4B^2	2	A^3B^2	2	A^4B^1
I(arg)	Wild	11	6	A^1B^1	1	A^2B^2	2	A^1B^2	2	A^2B^1
VIII	$A^1B^1 \times A^2B^2$	8	4	A^1B^1	1	A^2B^2	1	A^1B^2	2	A^2B^1
XIV	$A^1B^1 \times A^3B^3$	9	2	A^1B^1	2	A^3B^3	2	A^1B^3	3	A^3B^1
XXX	$A^3B^2 \times A^3B^3$	8	3	A^3B^2	2	A^3B^3	2	A^3B^3	1	A^3B^2
XXXI	$A^1B^1 \times A^4B^4$	9	2	A^1B^1	3	A^4B^4	2	A^1B^4	2	A^4B^1
XXXII	$A^3B^2 \times A^4B^4$	8	0	A^3B^2	4	A^4B^4	3	A^3B^4	1	A^4B^2
XXXIII	$A^6B^6 \times A^2B^2$	9	1	A^6B^6	0	A^2B^2	4	A^2B^6	4	A^6B^2
XXXVII	$(A^1B^2 \times A^1B^1) \times A^2B^1$	11	1	A^1B^1	6	A^2B^2	2	A^1B^2	2	A^2B^1

body XXXVII) was obtained from a cross with a flat mycelium (heterokaryon with common A factors). The f_1 mycelia from this cross could not be distinguished from wild type monokaryons, which shows that none of the characteristics of Flat are hereditary. The fact that all four expected mating types are obtained shows that the fruit was not parthenogenetic but that the

appropriate nucleus of the Flat mycelium migrated into the mate and true dikaryotization took place. Some mutants, or possible mutants, which were obtained from crosses in table 1 will be described below and are omitted from the table.

CROSSES WITH STREAK

The genetics of Streak was studied by analysis of both random spores and tetrads of spores from crosses of wild type monokaryons with Streak. The results of these two methods of analysis are recorded separately and they are pooled for calculation of linkage values.

TABLE 3

Random spores from crosses with Streak.

Fruit	Cross	f_1							
		Streak				Wild			
		A^1B^1	A^2B^2	A^1B^2	A^2B^1	A^1B^1	A^2B^2	A^1B^2	A^2B^1
II	$A^2B^1+ \times A^1B^2s$	9	0	0	0	0	4	1	7
III	$A^2B^1+ \times A^1B^2s$	4	0	4	0	2	0	0	0
IV	$A^2B^2+ \times A^1B^1s$ (F_1 from III)	1	2	4	1	2	3	0	2
V	$A^2B^1+ \times A^1B^2s$ (F_1 from III)	5	1	4	2	2	4	0	7
VI	A^2B^1+ (single hypha culture) $\times A^1B^2s$	4	2	4	1	0	4	0	7
XVII	$A^2B^1+ \times A^1B^2s$ (new Streak)	1	1	2	1	0	1	1	2
VII	$A^1B^1+ \times A^2B^2s$	2	5	0	6	5	2	5	0
Total		26	11	18	11	11	18	7	25

Total Streak = 66
Total wild = 61

Total parental (A & s) = 104
Total non-parental = 23

In all but two (fruiting-bodies XVII and XXIV) of the crosses with Streak, the Streak culture used was derived from an old original isolate obtained before the regular occurrence of Streak under appropriate conditions was suspected. This original Streak was obtained incidentally during the course of some early experiments in which the monokaryons A^1B^1 and A^1B^2, separately cultured in liquid media, were blended and plated out in large Petri dishes. These cultures were then irradiated with ultra-violet light of wave-length 2,537 Å for a dosage of 500–1000 ergs/mm^2. The aim was to obtain mutant A factors since it was thought that any such mutation would lead to rapid dikaryotization of the whole culture. The results were all negative as regards their primary objective and, in view of the properties of Flat, the validity of the technique is questionable. That the irradiation was irrelevant to the genetic properties of the Streak obtained follows from a compari-

son of the results from fruits XVII (random spores table 3) and XXIV (tetrads table 4) with those from other fruits.

The mating types of 127 mycelia, isolated as random spores from crosses with Streak are shown in table 3. Fruiting-body II, the first fruit analyzed, gave peculiar ratios and it was partly on this account that other, different, crosses were made and that tetrads were analyzed. The complete data show conclusively that Streak segregates in a normal Mendelian manner, independently of the *B* factor but linked to the *A* factor.

The numbers of different types of tetrads isolated from fruits of matings with Streak are given in table 4. In order to incorporate the results of different crosses into one table the allelic constitution of the members of each kind of tetrad has not been entered. Instead, classes of tetrads have been designated in a generalized form from which the allelic constitution of each

TABLE 4

Tetrads from crosses with Streak.

Loci		Classes of tetrads*										
A & *B*		2-P	2-P	2-P	2-N	2-N	2-N	4-	4-	4-	4-	4-
A & *s*		2-P	2-N	4-	2-P	2-N	4-	2-P	2-N	4-	4-	4-
B & *s*		2-P	2-N	4-	2-N	2-P	4-	4-	4-	2-P	2-N	4-
Fruit	Cross											
XXXIV	$A^1B^2s \times A^2B^1+$	0	0	2	0	0	1	0	0	0	0	1
XXXV	$A^1B^2s \times A^2B^1+$	0	0	0	1	0	0	2	1	1	0	1
XXIV	$A^1B^2s \times A^2B^1+$	0	0	1	0	0	1	5	0	2	0	0
XX	$A^2B^2s \times A^1B^1+$	1	0	0	0	0	0	2	0	0	0	0
XXXVIII	$a^8B^2s \times a^7B^1+$	0	0	1	0	0	0	3	0	0	0	0
XXXIX	$a^8B^2s \times a^7B^1+$	2	0	0	2	0	0	3	0	0	0	0

* 2-P = Two type parental
2-N = Two type non-parental
4- = Four type.

spore can readily be obtained for any particular cross. Thus in the first cross, $A^1B^2s \times A^2B^1+$, the class which is 2-type parental as regards the *A* and *B* factors, and 4-type as regards *A* & *s*, and *B* & *s*, includes tetrads of the constitution A^1B^2s, A^1B^2+, A^2B^1s, A^2B^1+. It will be noted that only 11 classes are entered although there are 12 possible kinds of tetrads. This is because the last class, which is 4-type for all three pairs of factors, includes tetrads of two kinds. In the case of the first cross, $A^1B^2s \times A^2B^1+$, for example, the two kinds are: A^1B^1s, A^2B^2s, A^1B^2+, A^2B^1+; and A^1B^1+, A^2B^2+, A^1B^2s, A^2B^1s. The distinction is one of chromatid segregation and is irrelevant to the present purpose. In eight cases only three mycelia were available for analysis, the fourth having failed to germinate or having been lost; the genotype of the missing sport was inferred in each case.

In the following calculations the results from fruiting-bodies XXXVIII and XXXIX in table 4 will be omitted. These fruiting-bodies were obtained

from crosses between "mutants" and it will be shown that the data from them cannot be considered homogeneous with the data from the other fruiting-bodies.

Summing the results from random spores and tetrads we get:

	Parental	Non-parental	P. on a 1:1 hypothesis
A & *B*	111	104	55%
B & *s*	110	105	68%
A & *s*	168	47	0.005%

From the results of the tetrad analyses it can be concluded that Streak segregates in a 1:1 ratio and since the ratio of Streak to wild type among random spores did not differ significantly from 1:1, no lethality is associated with Streak.

The map distance between *A* and *s*, on the basis of the combined data, is 47/215 = 22 units.

Further analysis of the tetrads shows the following:

	2-type parental	2-type non-parental	4-type	4-type %-
A & *B*	4	3	15	68
B & *s*	4	1	17	77
A & *s*	11	1	10	....

Only one 4-strand double crossover tetrad (2-N for A & s) was obtained out of the 33 tetrads analyzed.

Now the frequency of 4-type tetrads as between any two unlinked genes is a function of the frequency of crossing over between each of these genes and the centromeres of their respective chromosomes. This function is given by $T = a + b - 3ab/2$ (PERKINS 1949, WHITEHOUSE 1949a), where a and b represent the frequencies of second division segregation, in other words half the cross over values, neglecting multiple cross-overs, of the two genes concerned, and T is the frequency of 4-type tetrads.

Substituting the frequencies of 4-type tetrads for *A* and *B*, and for *A* and *s* we have

$$A' + B' - 3A'B'/2 = 0.68$$
$$\text{and } s' + B' - 3s'B'/2 = 0.77,$$

where A′, B′, and s′ represent the frequencies of second division segregation of the genes *A*, *B*, and *s* respectively.

The crossover value between *A* and *s* is 22 percent, doubling this value and expressing it as a frequency it is clear that either $A' + s' = 0.44$, or $|A' - s'| = 0.44$.

Of the three sets of three simultaneous equations so obtained only one set, where $A' - s' = 0.44$ has an acceptable solution. The values obtained are $A' =$

0.59, B′ = 0.8, and s′ = 0.15. The map distances of these three loci from the centromeres are therefore, according to these calculations, as follows:

The general solutions to these equations are given in Appendix 1. Their significance should not be overestimated. Apart from the very small numbers available in the present case, the errors of the solutions to the equations become greatly magnified in certain areas. Only in the case of the linkage value can double crossovers be included, they can be estimated, in this case, from the frequency of 2-N tetrads for the two linked genes (see PERKINS 1949).

To these tentative chromosome maps of Schizophyllum the locus " Knäuel-Fruchtkörper " controlling a recessive, dikaryon, character (ZATTLER 1924), can be added on the remaining, third (ERLICH and MCDONOUGH 1949) chromosome.

From fruiting-bodies XXXVIII and XXXIX the proportion of crossovers between *A* and *s* is 2/53 (tetrads and unpublished random spores). From the rest of the data the proportion is 47/215. This represents a discrepancy from homogeneity significant between the levels 0.01 and 0.001.

MUTATION OF THE INCOMPATIBILITY ALLELES

Over a period of two years about 400 spores have been isolated and tested for mating type. Of these about 100 were isolated in tetrads. Another 100 were tested by the use of only two testers, a method, described in an earlier section, which does not enable mutants to be detected.

Among the 300 spores in which mutants would have been detected, nine possible mutant *A* alleles and one possible mutant *B* allele were obtained. The term " possible mutant," as used here, indicates that the isolate dikaryotized testers having both *A* or *B* alleles, a phenomenon which would, no doubt, be classed as " illegitimate dikaryotization " by many authorities. Apart from any genetic evidence this possibility can be ruled out, in the present case, for two reasons. In the first place the behavior of pairs of mycelia with common *A*, and also with common *B*, factors is known, and contradicts the idea of " illegitimate dikaryotization "; and secondly, the clamp-connections in these exceptional matings did not differ from those found in other dikaryons. " Pseudo-clamps " are, indeed, found in Schizophyllum, but they do not appear to be of any special significance.

Some of the ten possible mutants were analyzed further: a^1, a^2, a^7, a^8, and b^1 were each mated with A^1, A^2, A^3, A^4, A^5, and A^6; and a^1, a^2, a^7, and a^8, were mated among themselves in all combinations. Only two identities $a^1 = a^7$, and $a^2 = a^8$, were discovered as a result of these matings. Since these cultures differed in mating type from all the stock cultures in the laboratory up to that time, the possibility of contamination can be eliminated, furthermore a^7 and a^8 were isolated in a tetrad.

Normal fruiting-bodies were obtained from the possible mutants a^1, a^2, a^6, a^7, a^8, and b^1 in matings with both of the testers that each dikaryotized.

Several f_2 mycelia from crosses involving a^1, a^6, a^7, and a^8, and f_3 mycelia from crosses involving a^1, a^7, and a^8, were analyzed. In each case the possible mutants were recovered and they segregated in a normal Mendelian manner. It is concluded that a^1, a^6, a^7, and a^8 are inherited as true mutant alleles with the reservation that the possibility of contamination has not been eliminated in the case of a^6. It can be assumed that some, if not all, of the other possible mutants are also inherited as true mutant alleles.

The mutant alleles a^7, and a^8, identical in mating characteristics with a^1, and a^2, respectively, are of special interest. They were obtained from the fruiting-body XXXV ($A^2B^1+ \times A^1B^2s$), and both appeared in the same tetrad, T39. Three of the spores in this tetrad had the following constitutions: $T39^1 = A^2B^2+$, $T39^2 = a^7B^1+$, $T39^3 = a^8B^2s$. The fourth spore was either lost or was not produced, it would presumably have had the constitution A^2B^1s or A^1B^1s.

Monokaryons with a^7 and a^8 were crossed (fruiting-bodies XXXVIII and XXXIX, table 4) and from them, besides the 11 tetrads shown in table 4, one further tetrad, T53, was found to contain two possible mutants. The constitution of the four spores from T53 was: $T53^1 = A^1B^1s$, $T53^2 = A^2B^2+$, $T53^3 = a^8B^1s$, $T53^4 = a^7B^2+$. From another cross of a^7 and a^8, this time between the two non-Streak mycelia $a^8B^2 \times a^7B^1$ four tetrads were analyzed. Three of these consisted of spores with the expected distributions of alleles, the fourth contained another possible mutant. One spore was missing from this fourth tetrad, the constitutions of the remaining three were: $T62^1 = a^7B^2$, $T62^2 = a^8B^1$, $T62^3 = A^1B^1$. Thus in both these cases the possible mutants from crosses of a^7 and a^8 proved to be the alleles A^1 and A^2 which were the alleles present in the cross from which the "mutants" a^7 and a^8 were obtained. The possibility of contamination in these cases is negligible owing to the technique used for tetrad isolation and also, in the case of T53, because of the fit of the *B* and *s* alleles in $T53^1$ and $T53^2$ with expectation.

That the occurrence of these mutants in tetrads T39, T53, and T62, is evidence for crossing over within the *A* "gene" will be elaborated in the last section of this paper. Further evidence lies in the fact that the "alleles" a^1 and a^2 were obtained from a cross of A^1 and A^2 ($A^1B^2s \times A^2B^1+$) as were the "alleles" a^7 and a^8.

SPONTANEOUS DIKARYON FORMATION

In the course of the present work six mycelia, each believed to be a normal monokaryon, have produced clamp-connections and have given rise to fertile fruiting-bodies. Five of these were from the wild fruiting-body XV(ad) of constitution $A^3A^4B^3B^4$, and the sixth was from XXXII ($A^2B^2 \times A^6B^6$).

Of ten spores isolated from XV(ad) one (305) was found to have produced clamp-connections when first examined, by which time it was about 8 cm in diameter. Stock cultures 301, 307, 308, and 309, constituting the four mating types from this fruiting-body, were kept in duplicate, one set at room

temperature, and the other at 5°C. Within three weeks all four cultures at room temperature had clamp-connections and subsequently gave rise to apparently normal fruiting-bodies.

Two of these cases of spontaneous dikaryon formation were further analyzed. The mycelium 305 was found, by the use of di-mon matings (see PAPAZIAN 1950c), to have the constitution $A^3b^2 + A^4B^4$, and the spores from its fruit fell into the four classes A^3B^4, A^4b^2, A^3b^2, and A^4B^4. The apparently new allele b^2 was not further studied. The stock mycelium 301 which when first isolated was of mating type A^3B^3 gave rise to spores of mating types A^3B^3, A^4b^3, A^4B^3, A^3b^3. The apparently new allele b^3 was found to be identical in mating properties with B^1, an allele found in the wild fruiting bodies I(arg), and XXV(mad). Contamination in this case is ruled out since no mycelium or spore having the constitution A^3B^1, or A^4B^1, had been available in the laboratory up to that time. Thus in both cases analyzed a new B factor not present in the stock was found, but both A factors were identical with those present in the stock. This corresponds exactly to a similar case of spontaneous dikaryon formation analyzed by KNIEP (1929).

TABLE 5

Analysis of mycelia and sectors from three spores of the aberrant tetrad T 20.

		A^1B^1	A^2B^2	A^4B^3	A^2B^1	Constitution
Dikaryotic sector	T20¹	H	H	N	N	$A^1B^1 + A^2B^2$
	T20²	H	H	N	N	$A^1B^1 + A^2B^2$
	T20³	N	N	H	H*	$A^1B^2 + A^2B^1$
Streak mycelium before appearance of sector	T20¹	−/+	−/−	−/+	−/−	?
	T20²	−/+	−/−	−/+	−/−	?
	T20³	−/+	−/−	−/+	−/−	?
Streak mycelium after appearance of sector	T20¹	−/+	−/−	−/−	−/−	A^2B^2
	T20²	−/+	−/−	−/−	−/−	A^2B^2
	T20³	−/−	−/−	−/+	−/−	A^2B^1

* H = Hemi-compatible di-mon
N = Non-compatible di-mon (see PAPAZIAN 1950c)

One further, more complex, case of spontaneous diploidization is worthy of record. From the fruiting-body XX ($A^2B^2s \times A^1B^1+$), besides three perfectly regular, complete, tetrads, a fourth aberrant tetrad was isolated. This aberrant tetrad (T20) gave rise to three mycelia which appeared, initially, to be Streaks. Each exhibited, however, dikaryotizing properties that would be expected of a Flat mycelium of constitution $A^2B^2 \times A^2B^1$. Later each of the three cultures produced an apparently typical dikaryotic sector. Subcultures of the Streak and the dikaryotic portions of each of the three cultures remained constant. The analysis of their mating types is given in table 5. No fruiting-bodies could be obtained from the dikaryotic sectors or from mycelia diploidized by the "Streak" sectors. No subsequent sectors appeared in the "Streak" cultures, although they were sub-cultured for

three successive transfers. No explanation for the aberrant behavior of the mycelia from T20 is offered.

DISCUSSION

The data described in the preceding sections present three main problems. The fact that Streak behaves as a single gene mutant raises the question of the control of unilateral dikaryon formation, a property common to both Streak, and Flat (heterokaryons with common *A* factors), and also the question of the origin of Streak mutants from Flat mycelia. The data concerning "mutant" incompatibility factors suggests that the incompatibility factor is not governed by a single gene, as is commonly believed, but by two or more closely linked genes. These topics will be elaborated in turn.

For GREIS (1942) the significance of unilateral dikaryon formation lies in the support that it provides for HARTMANN's (see 1943) theory of sex. It is proposed here to emphasize another aspect of unilateral dikaryon formation, namely, as evidence that the immediate control of dikaryon formation must lie in the cytoplasm. Consider a mating such as $A^1B^1s \times A^2B^2+$. It is known that these two types of nuclei can exist together as a dikaryon since they do so, regularly, in the A^2B^2+ mycelium. It is also known that the A^2B^2+ nucleus is capable of migrating into another mycelium. That it does not, in fact, migrate into the A^1B^1s mycelium and form a dikaryon almost certainly means that the A^1B^1s cytoplasm inhibits either the entry of the A^2B^2+ nucleus, or its multiplication once it has entered. The results of experiments with Streak, and Flat (PAPAZIAN 1950c) indicate that in both cases it is the entry of the compatible nucleus that is inhibited.

What light, if any, does the phenomenon of unilateral dikaryon formation throw on the nature of the incompatibility factors? There are insufficient data on which to found a theory but, following T. H. Huxley ". . . consecutive wrong-thinking is the next best thing to right thinking," certain speculations may be excused.

The first and least speculative postulate is that the incompatibility factors actuate some kind of "trigger" mechanism while the fundamental factors controlling the processes leading to the formation of a fruiting-body are associated with other genes. This is likely on general grounds and is supported by the fact that monokaryons do sometimes produce fruiting-bodies parthenogenetically which are normal except that they produce basidiospores of only one mating type (KNIEP 1928). It seems likely, furthermore, that the contributions of the *A* and *B* factors differ only in degree, at least in Schizophyllum (but see FULTON 1950), since the similarity between Flat and the barrage region in morphology and physiologically is quite apparent. Let it be assumed then, that the "trigger" mechanism is released by a reaction between two complementary substances, one of which is associated with the nucleus, the other with the cytoplasm. Thus, whenever the nucleus of a mycelium comes into contact with the cytoplasm of a different, compatible, mycelium, this reaction takes place. Both nuclear and cytoplasmic substances

must be considered to be ultimately under genic control. Unilateral dikaryon formation would result from a failure of the reaction to take place in one of the mycelia. This failure might be due to a genic deficiency, resulting in a lack of the cytoplasmic substance, or it might be due to the previous occurrence of a similar reaction in the mycelium with consequent depletion of the cytoplasmic substance. The first of these possibilities would account for the behavior of Streak, and the second would account for the behavior of Flat.

An analogy for such a scheme can be found in the agglutination reactions of the red corpuscles of a person in the serum of a different individual and in the fixation of a third substance (*vide* inhibitor) in antigen-antibody reactions.

The formation of heterokaryons such as Flat would represent a partial reaction, allowing the entry and multiplication of nuclei but not conjugate nuclear division, clamp-connection formation, and the formation of fruiting-bodies.

It is of interest that in those species of ascomycetes which have sexual organs, heterokaryosis takes place between mycelia of similar mating type but not between compatible mycelia (WHITEHOUSE 1949b). Although there is some evidence that heterokaryons are not formed between mycelia of the same mating type in Schizophyllum, the underlying mechanism is probably quite different to that in the ascomycetes in which there are only two alternative sexual types.

The fundamental similarity between Streak and Flat is that they both dikaryotize unilaterally, and the fundamental difference between them is that in the one case this character is hereditary and in the other it is not. Before discussing the origin of Streak from Flat it is useful to review the studies of QUINTANILHA (1938, 1940) on certain dwarf mycelia of *Coprinus fimetarius*. These dwarf mycelia all dikaryotized unilaterally and they never showed clamp-conections or fruited. The interesting feature of these dwarfs in the present connection is that in some of them the character was hereditary and in some others it was not. Thus in a mating $A^1B^2 \times A^2B^2$ ($Ab \times ab$ in the original nomenclature), nearly all of the progeny were dwarfs and non-fertile; one spore, however, was wild type and proved to have a mutant A allele. This mutant was mated with a monokaryon A^2B^1 and the resulting fruit gave rise to wild types and dwarfs. In the latter the dwarf character was not associated with any particular mating type. *Dwarf* × *dwarf* matings were sterile but *dwarf* × *wild type* matings gave rise to normal fruiting-bodies whose spores were all wild type. The dwarf character in this case was, therefore, not hereditary. In his later studies QUINTANILHA (1940) obtained 200 tetrads of basidiospores, from a certain fruiting-body, of which 100 proved to be composed of 2 *dwarfs* + 2 *wild type*. The remaining 100 tetrads had various lethals. The dwarf character was again independent of mating type but the character was in this case hereditary.

In any attempt to account for the origin of Streak from Flat, three possibilities have to be considered. Firstly, the spontaneous mutation rate to Streak

under any conditions may ensure that Streak nuclei are present in all monokaryons, and the special conditions under which Streak mycelia appear may act selectively; secondly, these special conditions may act as a general mutagenic agent, selection being of little or no import; the third possibility is that the special conditions induce a directed mutation to Streak. Further experiments are in progress to distinguish among these alternatives; from the present data, however, some theoretical possibilities can be evaluated.

No direct estimate of the frequency of spontaneous mutation to Streak can be made on the basis of the small number of single-spore cultures that have been examined. In vegetative mycelia, where the number of nuclei is very large, mutations to Streak would only be discernible if the mutant nuclei multiplied and sectored out. It is known, however, that Streak is as viable, and grows at the same rate as, a wild monokaryon or dikaryon, therefore any Streak hyphae in such mycelia might be expected to develop into sectors which would be detected. It is unfortunate that Streak nuclei cannot, apparently, be introduced experimentally into Flat mycelia, dikaryons, or into monokaryons of their own mating type, since this would provide direct evidence on the ability of Streak nuclei to multiply and sector out in such mycelia. The difference in appearance between Streak growths in blends to which Streak nuclei have, and have not (PAPAZIAN 1950c), been deliberately added provides further evidence that Streak nuclei are not present before the blend is made.

The evidence concerning a selective advantage of "streak" in old cultures of Flat is contradictory. A selective disadvantage is indicated by the fact that Streak grows less rapidly than Flat in acid media which is the usual reaction of old cultures of Flat. That new growths of Streak do, in fact occur in old cultures in which growth of Flat has apparently ceased is evidence, on the contrary, that Streak has a selective advantage.

In general the present limited data indicate that the mutant Streak nuclei are not present before the blending of Flat and the most plausible explanations to account for the origin of new growths of Streak are: that an old Flat culture acts as a general mutagenic agent and that these conditions also offer a selective advantage for Streak over other mutants, or that the special conditions induce a directed mutation to Streak.

The level at which selection may be operative is a central problem in the analysis of the origin of Streak. The idea of selection operating at the nuclear level is not new (PONTECORVO 1946) but it is pertinent to consider the extreme limits at which selection may be effective. Most superficially selection might be effective in preventing or favoring the formation of sectors, *i.e.*, a certain percentage of nuclei in all mycelia are Streak, and these multiply at a rate comparable to that of wild type nuclei, but they do not sector out unless the special conditions are operative. Alternatively, there may be a certain spontaneous mutation rate to Streak in all mycelia but the mutant nuclei multiply at a very low rate, or not at all, unless the special conditions are operative. If the stage at which selection acts be still earlier, one can imagine the

mutant nuclei dying as soon as they are formed. Although a theoretical distinction can still be made between the situation in which a gene mutates but is immediately eliminated and that in which the gene does not mutate, the technical difficulties involved in making a practical distinction may prove very exacting. This difficulty should not be confused with the difficulty of distinguishing between induced mutation and selection in bacterial "adaptation" discussed by JACKSON and HINSHELWOOD (1950).

The frequency with which mutation of the incompatibility alleles takes place is a matter of considerable interest. As a result of his studies on *S. commune*, KNIEP (1928) put forward the opinion that mutation occurs frequently during meiosis but only rarely in vegetative mycelia, that mutation to the same alleles occurs frequently, and that back mutations occur frequently. The present data generally support these contentions. The question of mutation rate in vegetative mycelia is difficult to assess. It can safely be assumed, however, that if a mutation of a *B* factor were to occur in a monokaryon, a Flat culture would result and would be detected. Similarly a mutation of the *A* factor would produce sectors of different mating types. Since neither of these events have been observed in the course of the present work, and in view of the relatively enormous number of nuclei present in the average mycelium, mutation of the incompatibility factors in vegetative mycelia must be considered to be much less frequent than the present data indicate for the fruiting-bodies.

The three contentions of KNIEP already suggest that the so called "mutant alleles" are not true mutants at all but that new factors are produced by crossing over. The occurrence of "mutants" a^7 and a^8 confirms the suggestion that there are at least two closely linked genes associated with the *A* incompatibility factor, and that a different allele at any locus in the group provides a different incompatibility factor. The group of *A* loci thus behave physiologically as a single factor but must be considered as separate genes genetically. The term "A^1 factor" may still be used, but the term "A^1 allele" is a misnomer and should be replaced as soon as more data are available by symbols for the several alleles.

If two of the alleles for the A^1 incompatibility factor are $A_1{}^1$ and $A_2{}^1$ and for the A^2 factor $A_1{}^2$ and $A_2{}^2$, then in T39 crossing over occurred between these two loci producing $A_1{}^1A_2{}^2$ and $A_1{}^2A_2{}^1$ which correspond to factors a^7 and a^8. The missing spore in the tetrad would be A^1B^1s. In tetrads T52 and T63 crossovers occurred between $A_1{}^1A_2{}^2$ and $A_1{}^2A_2{}^1$ reconstituting $A_1{}^1A_2{}^1$ and $A_1{}^2A_2{}^2$ which correspond to factors A^1 and A^2. The missing spore in T63 would be A^2B^2.

KNIEP (1929), using the same organism, but unfortunately without being able to isolate tetrads, obtained in one instance a similar result. From a fruiting-body of constitution *AaBb* he obtained, in addition to the four types *AB*, *Ab*, *aB*, *ab*, two cultures with mutant *A* alleles: A_1B, and a_1b (KNIEP's nomenclature). These two mycelia were crossed and, in addition to the expected types A_1B, A_1b, a_1B, and a_1b, he obtained the two original alleles *A* and *a*. It is not certain that KNIEP's *A* and *B* factors correspond to my *A* and

B factors since KNIEP did not distinguish the Flat and Barrage reactions. In view of the much higher number of *A* " mutants " than *B* " mutants " that he obtained it is likely that his factors do correspond to mine.

It is curious that the combination $A_1{}^1A_2{}^2$ and $A_1{}^2A_2{}^1$ seems to inhibit crossing over between this region and the *s* locus.

Groups of distinct genes which act more or less as a unit physiologically have been reported in various other instances. In some of these, such as those in Drosophila (E. B. LEWIS 1945; GREEN and GREEN 1950) and maize (LAUGHNAN 1949) crossing over has been detected and the individuality of the genes has been established. In other cases such as the Rhesus factor in man (FISHER 1947) and Neurospora (BONNER 1950), and the incompatibility (D. LEWIS 1949), and anthocyanin (SILOW and YU 1942), factors in higher plants, crossing over has not been demonstrated and the question as to whether the factors represent several, linearly arranged, genes; or independently mutating parts of a single, two- or three-dimensional, gene, remains open.

In the present instance a change in any allele in the *A* incompatibility group provides a functionally different incompatibility factor. The resulting pattern of compatibility of the *A* factors, neglecting the *B* factors, is given in table 6.

TABLE 6

Pattern of compatibility with various combinations of alleles in the A group.

	$A_1{}^1A_2{}^1$	$A_1{}^1A_2{}^2$	$A_1{}^2A_2{}^1$	$A_1{}^2A_2{}^2$
$A_1{}^1A_2{}^1$	−	+	+	+
$A_1{}^1A_2{}^2$	+	−	+	+
$A_1{}^2A_2{}^1$	+	+	−	+
$A_1{}^2A_2{}^2$	+	+	+	−

The number of different factors in each of the incompatibility groups, *A* and *B*, has been roughly estimated at 100 (WHITEHOUSE 1949a). These could be accounted for by ten alleles at each of two loci, or about five alleles at each of three loci, or by other combinations. The relatively small number of different factors found in the gasteromycetes (WHITEHOUSE 1949a), which is incongruous from an evolutionary point of view since the efficiency of the multiple-allele incompatibility system as an outbreeding mechanism is thereby greatly diminished, can be explained by the existence of a smaller number of loci for incompatibility in the gasteromycetes, limiting the possible number of factors.

From an evolutionary point of view there are certain advantages in having several rather closely linked genes controlling each of the incompatibility factors.

Multiple-allele heterothallism as an outbreeding mechanism, assuming that outbreeding is itself advantageous, is more efficient the greater the number of different factors present. In the tetrapolar basidiomycetes the number of different incompatibility factors that can be maintained in a population is a

function of the population size and structure, and the mutation pressure, as is true in the flowering plants (WRIGHT 1939). New factors have a selective advantage which is an inverse function of their frequency (Appendix 2), and factors are lost by random sampling. The number of factors in any given population represents an equilibrium between these two processes. Actually there is a possibility, in the case of Schizophyllum, that the factors are not entirely equivalent among themselves (PAPAZIAN 1950c). Particular factors may have a selective advantage over others which would tend to decrease the number of different factors maintained.

Mutation of an individual gene in the incompatibility group, an event which there is no reason to believe is more frequent than in other genes, produces not only a new factor initially but produces the potentiality of a whole series of new factors by crossing over and recombination with different alleles of the other genes in the group. Until a new allele is in equilibrium in a population, crossing over can thus be considered equivalent to mutation of the factor to a new factor. A certain minimum crossover rate would thus be advantageous. There is, however, another factor which would come into play with high rates of crossing over or with independent segregation. The fertility of crosses of monokaryons from the same fruiting-body would be increased (Appendix 3) and thus the amount of inbreeding would be increased. This factor would probably be negligible for values of the order of one percent. In certain situations where dissemination of spores is very poor and the spacing of fruiting-bodies very wide, an increase in fertility among spores from the same fruiting-body might conceivably be advantageous, but in general it is more probable that a minimum of inbreeding and a maximum of outbreeding are the most advantageous conditions. These would be favored by the genes in each incompatibility group being closely, but not too closely, linked.

The rather frequent occurrence of spontaneous dikaryon formation cannot be accounted for on the basis of multiple incompatibility loci unless by chromosomal interchange at the *A* and *B* regions, which, without supporting evidence, seems unlikely. In the only case analyzed the *A* factor which appeared spontaneously was the same as one of the *A* factors in the parent fruiting-body but the *B* factor which appeared was new. It is of interest that KNIEP (1929) reported an identical case: a fruiting-body *AaBb* gave rise to a monokaryon *Ab* (KNIEP's nomenclature) which became a dikaryon spontaneously, this dikaryon fruited and proved to be of constitution *AaBb* where *B* was a new factor. This suggests that in these aberrant monokaryons the second *A* factor, but not the *B* factor is somehow present though masked.

SUMMARY

1. Evidence is presented that the *A* incompatibility factor, which functions as a single unit physiologically, is controlled by at least two closely linked genes. The evolutionary consequences of this finding are discussed.

2. An aberrant type of mycelium, Streak, which has been shown, in a previous publication, to be produced regularly under certain special condi-

tions, is found to be controlled by a single gene. This gene is linked to the *A* incompatibility factor and is independent of the *B* factor. Possible modes of origin of this mutant are discussed.

3. Formulae for the mapping of loci in unordered tetrads, for the selective advantage of new incompatibility factors, and for the increase of inbreeding due to mutation of the incompatibility factors are given.

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APPENDIX 1

Unordered tetrads with three factors two of which are linked

Let x and y = The second division segregation frequency of the two linked factors.
let z = the second division segregation frequency of the independent factor.
let q and r = the frequencies of four-type tetrads of each of the linked factors with the independent factor respectively.
let S = The recombination frequency between the two linked loci.

Then $q = y + z - 3yz/2$

$r = x + z - 3xz/2$

and $2S = x + y$ or $|x-y|$

If we let $\alpha = q - r$

$\beta = \alpha - 2S$

$\gamma = -\alpha - 2S$

and $\delta = q + r$ Then the solutions for x, y, & z, are:

$2S =$	$x+y$	$x-y$	$y-x$
$x =$	$2\frac{3Sr+\beta}{3\delta-4}$	$\frac{1}{2}\frac{3Sr+\gamma}{-\alpha}$	$\frac{1}{2}\frac{3Sr+\beta}{\alpha}$
$y =$	$2\frac{3Sq+\gamma}{3\delta-4}$	$\frac{1}{2}\frac{3Sq+\gamma}{-\alpha}$	$\frac{1}{2}\frac{3Sq+\beta}{\alpha}$
$z =$	$\frac{\delta-2S}{2-3S}$	$\frac{-\alpha}{3S}$	$\frac{-\beta}{3S}$

These solutions are subject to the error that two of the equations are in terms of second division segregation frequencies and the third is in terms of recombination frequencies.

In the case of two factors lying in the same chromosome arm if x′ represents the frequency of single chiasmata between the centromere and the factor lying nearest the centromere and y′ represents the frequency of single chiasmata between the two factors,

then $x = x'$

and $y = x'(1-y') + y'(1-x') + \frac{1}{2}xy$

so $y-x = y' - \frac{1}{2}x'y'$

which is not equal to twice the recombination value even when the latter is corrected (PERKINS, 1940) for double crossovers.

A further error in these equations which applies equally to the analysis of three independent loci in unordered tetrads (WHITEHOUSE 1950b) and to chromosome maps based on segregation values in ordered tetrads such as Neurospora, is that the results are in terms of second division segregation frequencies. Second division segregation frequencies are, in fact, a worse guide to map distance than the crude recombination frequencies. Assuming absence of interference, the frequencies of 0, 1, 2, 3, multiple chiasmata are distributed according to the Poisson series

$$e^{-m}\left(o,\ m,\ \frac{m^2}{\underline{2}},\ \frac{m^3}{\underline{2}}\cdots\right)$$

(HALDANE 1931), where m = mean No. of chiasmata. In the case of recombination one chiasma or any number of multiple chiasmata give 50 percent recombination. The number of such single and multiple chiasmata is $1-e^{-m}$ and the recombination value $1/2\ (1-e^{-m})$. The segregation values, on the other hand, are $2/3[1-(-1/2)^n]$ where n = the number of chiasmata occurring simultaneously (MATHER 1935). The second division segregation value is thus

$$\Sigma e^{-m}\tfrac{2}{3}\frac{m^n}{\underline{n}}\left[1-(-\tfrac{1}{2})^n\right]=\tfrac{2}{3}e^{-m}(e^m-e^{-m/2}).$$

and the map distance calculated from such data is half of this value.

Figure 1 shows graphs of map distance as a function of chiasmata frequency assuming complete interference and based on recombination, and second division segregation, assuming that chromosome and chromatid interference are absent.

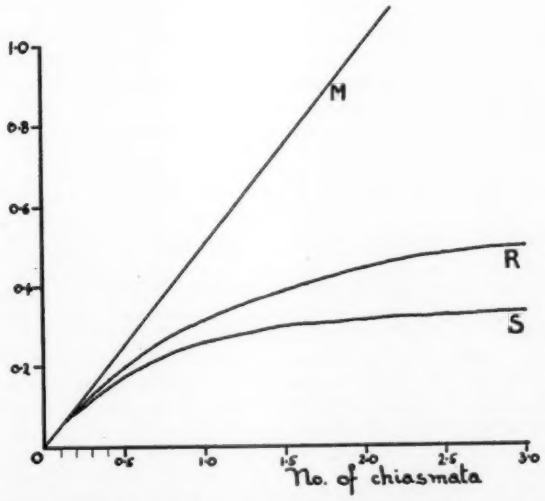

FIGURE 1.—Graphs showing relationship between mean number of chiasmata and map distance. With complete interference (top line); with no interference and map distance based on recombination (middle curve), and segregation (lower curve).

APPENDIX 2

Selective advantage of new incompatibility factors

Let A^m = the new factor
let q = the frequency of A^m
so $1-q$ = the frequency of all other factors.
let n = the number of different factors in each of the A and B series.
Then the frequency of matings without A^m

$$(X) = (1-q)^2\ n^2 - 2n/n^2$$

and the frequency of matings heterozygous for A^m

$$(Y) = 2[q(1-q)\ n^2 - n/n^2]$$

the frequency of matings homozygous for $A^m = 0$.

If q' = the frequency of A^m in the next generation, then

$$q' = \frac{\frac{1}{2}Y}{X+Y} = \frac{q(n^2-n)}{n^2(1+q)-2n}$$

Note: when $q = 1/n$, then $q' = q = 1/n$.

APPENDIX 3

Inbreeding and mutation of the incompatibility alleles

Let F = frequency of fertile matings among the spores of a fruit taken in pairs.
let m = mutation rate to the same new factor

Then the frequency of matings without mutant $= (1-m)^2/4$ and the frequency of matings heterozygous for mutant $= m(1-m)$ and

$$F = \frac{1+2m-3m^2}{4}$$

Note: when $m = 0$, $F = 1/4$. F reaches a maximum of 1/3 when $m = 1/3$.

If m_1 = the mutation rate to different factors then

$$F = \frac{1+2m_1-m_1^2}{4}$$

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FORWARD AND REVERSE MUTATION IN A HISTIDINE-REQUIRING STRAIN OF ESCHERICHIA COLI[1]

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THERE is an increasing volume of evidence that bacteria resemble higher organisms in their growth and reproduction (LURIA 1946; LEDERBERG 1948, 1949; BOIVIN 1950; GOTTSCHEWSKI 1950). Their small size and rapid multiplication make bacteria ideal material for investigations of spontaneous and induced mutations, and of the relationship between the gene and its intracellular and extracellular environment. In the present study, a comparison is made in *Escherichia coli* between the rate of mutation of a form having no special growth factor requirements to a form requiring histidine for growth, and the rate of reverse mutation resulting in the restoration of the prototrophic condition. These mutation rates have been studied during the normal growth of the culture (spontaneous mutation) and after treatment with ultraviolet radiation. The experiments were also designed to detect related phenomena such as phenotypic lag and nuclear segregation. Although this study was limited to a single character, in the absence of recombination in this strain (LEDERBERG 1947) it is not possible to determine the exact genetic nature of the mutations involved.

Because of the technical difficulties, there have been few comparisons of forward and reverse mutation rates. GOWEN (1941) found that under the influence of X-rays, the ordinary tobacco mosaic virus apparently mutated to aucuba with a higher frequency than aucuba reverted to wild-type. In Serratia, BUNTING (1946) determined a rate of 10^{-4} per bacterium per generation for spontaneous mutation from dark red to bright pink, and a spontaneous reversion rate of 2×10^{-3} per bacterium per generation. Comparisons of X-ray-induced forward and reverse mutation rates have been made for individual genes in Drosophila (PATTERSON and MULLER 1930; TIMOFEEFF-RESSOVSKY 1934) but no consistent trend for mutation to occur in either direction has been established.

A quantitative study of mutations induced in bacteria by different conditions has been made by NEWCOMBE (unpublished). Comparing the effects of spontaneous, ultraviolet- and X-ray-induced mutation, NEWCOMBE found that the ratio of mutants resistant to phage T1 to streptomycin-resistant mutants was 1:1500 for spontaneous mutation, 1:10 and 1:40 for mutation after ultraviolet and X-ray treatment. In studies of streptomycin resistance and

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dependence DEMEREC (1950) found that strains of *E. coli* with high and low rates of spontaneous mutation retained their relative mutability when treated with ultraviolet radiation. Additional evidence is required to determine whether the spontaneous mutability of a character is an indication of its ability to be mutated by ultraviolet radiation or other agents.

In their studies of phenotypic or "phenomic" lag, DEMEREC and LATARJET (1946), NEWCOMBE (1948) and NEWCOMBE and SCOTT (1949) have shown that in strain B/r the majority of both spontaneous and induced mutations to T1 resistance are not expressed until two or three additional cell divisions have taken place. After ultraviolet treatment there are apparently some "zero-point" resistant mutations which do not require cell division for expression, but these form only a small fraction of the total number of mutants. No phenotypic lag has been detected for the spontaneous mutation of streptomycin resistance in the same *E. coli* strain (NEWCOMBE and HAWIRKO 1949) while several hours' growth was necessary for the expression of all X-ray and ultraviolet-induced mutations (NEWCOMBE, unpublished). DAVIS (1950) has reported that cell division is required for the expression of several types of ultraviolet-induced auxotrophic and prototrophic mutants.

MATERIALS AND METHODS

The strains used in the following experiments were derived from a histidine-requiring (h^-) mutant (No. 148-334) of strain 15 secured after X-irradiation by DRS. J. O. LAMPEN and R. R. ROEPKE of the AMERICAN CYANAMIDE COMPANY. RYAN (1948) has shown that h^- mutates spontaneously to histidine-independence (h^+) and application of the variance analysis (LURIA and DELBRÜCK 1943) to data obtained in connection with the present investigation indicates that the mutation of h^+ to h^- also occurs spontaneously. In one of the serially transferred cultures of h^- maintained for many months by RYAN and SCHNEIDER, a type of h^- distinguishable from the parent type by its phage sensitivity pattern was found. From this h^-, h^+ strain "15-2" was obtained by spontaneous mutation; the h^- strain "A-11" was derived from a spontaneous h^- mutant selected from 15-2 by the penicillin technique. Strain 15-2 was used for all the studies of mutation from h^+ to h^- and A-11 in all the reversion experiments. Both strains have a division time of about 45 minutes in aerated synthetic medium at 37°C. Two h^- strains unable to ferment lactose (Lac^-) were also used: Lac^-A, recovered from 148-334 by DR. J. LEDERBERG after ultraviolet treatment, and Lac^-II, isolated from A-11 after ultraviolet treatment. Stocks were maintained at about 5°C on slants of 1 percent nutrient broth plus 2 percent agar and transferred about every four months.

For all the experiments to be described, bacteria were grown in a "minimal" medium composed of a mixture of reagent-grade salts described by GRAY and TATUM (1944). Trace elements and asparagine were omitted. Cultures grown in this medium with the addition of 0.05 percent glucose are limited in size by the carbon source rather than by pH and rapidly resume

growth when transferred to fresh medium. In experiments where a dense suspension of bacteria in the logarithmic phase of growth was desired, the phosphate concentration was increased four times, and the glucose concentration ten times. The plating medium contained Gray and Tatum's salts with the addition of 0.5 percent glucose and 1.5 percent washed agar (RYAN and SCHNEIDER 1949). 25γ per cc of histidine monohydrochloride $\cdot H_2O$ was added to the medium in which h^- cells were grown, unless otherwise noted. Cultures were washed in 12-cc centrifuge tubes by resuspending three times with sterile 0.9 percent saline. Sterile distilled water was used for all dilutions. Liquid cultures for inocula were grown in tubes containing 10 cc of medium which were aerated by continuous rolling at 37°C. Individual cultures for experiments on spontaneous mutation rates were inoculated with about 50 newly grown cells. Large cultures for irradiation experiments were aerated at 37°C on a Warburg shaker. For reproducibility, cultures in the logarithmic phase of growth were used in all the experiments to be described.

CALCULATION OF MUTATION RATE

Forward and reverse mutation rates were calculated by three different methods. The derivations of the equations and methods for the calculation of the variance of each method are contained in the papers of LURIA and DELBRÜCK (1943), NEWCOMBE (1948) and LEA and COULSON (1949). All the methods involve the determination of the number of mutants in each of several cultures of similar size. By Method I, the average number of *mutations*, m, per culture is calculated from P_0, the proportion of cultures with no mutants:

$$P_0 = e^{-m} \qquad \text{I}$$

By Method II, m is obtained from r, the average number of mutant bacteria per culture and C, the number of similar cultures using the estimate

$$r = m \cdot \ln(Cm) \qquad \text{II}$$

to plot a family of curves relating r to m for different values of C. The average number of mutations may be estimated with greater statistical efficiency from r_0, the number of mutants in the median culture of the series using the equation

$$\frac{r_0}{m} - \ln m = 1.24 \qquad \text{III}$$

To obtain the mutation rate, the number of mutations per culture is divided by n/ln 2, n being the total number of cells per culture (NEWCOMBE 1948). Because of the high variance expected when m is estimated by Method II, conclusions as to mutation rates have been based on Methods I and III. However, Methods II and III give very similar mutation rates when applied to the date below or to the phage-resistance data published by NEWCOMBE (1948, table 1). These experiments were apparently not extensive enough to include cultures containing large clusters of mutants arising from exceptionally early mutations; such clusters lead to the calculation of excessively high mutation rates by Method II.

There are assumptions inherent in all three methods, and in each series of experiments controls were set up to test them. It is necessary to verify the expectation that mutations occur at random with a fixed probability. Since a very large series of similar cultures were not run, it is not practicable to compare the observed frequency of cultures with 1, 2, etc., mutants with the expected frequency. If, however, the mutation rate as calculated by Method I is constant regardless of culture size, one can assume that the first mutation in each culture, although not necessarily all subsequent mutations, occurs with a fixed probability. Methods II and III will give erroneous mutation rates unless new mutants divide at a rate equal to the division rate of the parental cells. If g equals the number of generations of growth after the appearance of the first mutation, the number of mutants at any time should

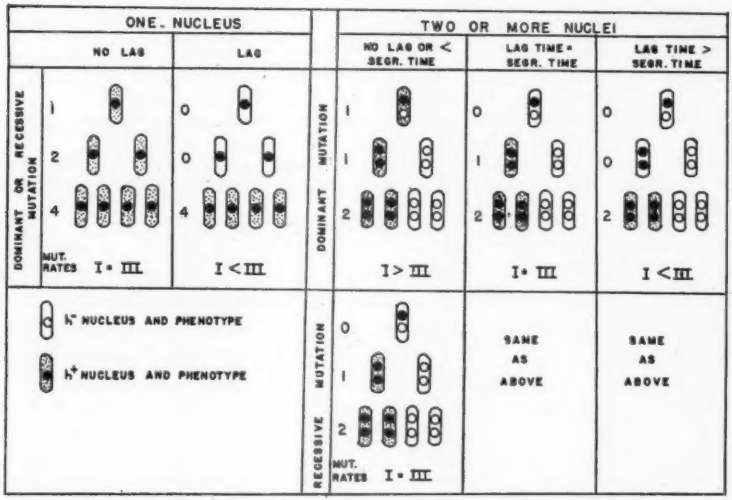

FIGURE 1.—Mode of increase of phenotypically mutant cells in mutant clones.

be $(g+1)2^g$ while the number of parental cells has increased by the factor 2^g. The effect of phenotypic lag on the mode of increase in detectable mutants within a single clone is illustrated in figure 1. If the bacteria are multinucleate, the calculated mutation rates will usually be lower than the true figure. Delay in phenotypic expression in either mono- or multi-nucleate cells will be reflected in differences between mutation rates calculated by Method I and rates obtained by Methods II and III and the duration of the period of phenotypic lag can be estimated from the extent of the discrepancy.

SPONTANEOUS REVERSION OF h^- TO h^+

Small cultures are required for the determination of mutation rates by Method I since some of the cultures must contain no mutants. Therefore, h^- cells were inoculated into small tubes containing medium in which the

amount of histidine limited the final size of the population to the desired level. The h^+ mutants which arose in some of the cultures continued to multiply, eventually producing turbidity in the culture tubes. Although one h^+ cell will multiply to give visible turbidity in 24 hours or less under the experimental conditions (RYAN and SCHNEIDER, personal communication), the number of turbid tubes continues to increase over a period of several days without an increase in the number of viable parental cells. Only the cultures becoming turbid within 24 hours after the end of h^- growth were counted. Table 1 lists experiments performed by the tube turbidity method, and includes data obtained by RYAN and SCHNEIDER (in press). The mutation rates as determined by Method I on cultures varying in size from 1.0×10^6 to 9.5×10^7 do not appear to be correlated with culture size ($t = .816$, D.F. = 11, $p = .4$). Thus, population size does not affect the probability of appearance of the first mutant in each culture.

TABLE 1

Estimation of mutation rate from h^- to h^+ using Method I.

	N Number of cultures	n h^- cells per culture $\times 10^7$	P_0	a Mutation rate $\times 10^{-8}$	Standard deviation* $\times 10^{-8}$
1	55	.10	.962	1.29	1.40
2	32	.17	.906	4.14	2.29
3	69	.22	.855	4.99	1.59
4	198	.41	.828	3.18	.59
5	69	.56	.793	2.86	.77
6	14	1.24	.571	3.12	1.28
7	60	1.30	.583	2.87	.58
8	57	2.40	.579	1.57	.33
9	26	3.04	.269	2.98	1.06
10	59	3.20	.390	2.03	.35
11	59	6.30	.068	2.94	.53
12	48	6.32	.208	1.72	.30
13	64	9.48	.016	3.01	.73
			Weighted average	2.86×10^{-8}	
			Standard deviation	1.77×10^{-8}	

* Standard deviation after LEA and COULSON (1949). These estimates of S.D. do not take into account the variance due to diluting and plating errors and are thus lower than the S.D. of the weighted average.

For the determination of the number of h^+ mutants in larger populations, cultures were grown in medium containing an excess of histidine. When the population had reached the desired size, the cells were washed and the entire cultures plated on minimal agar. Table 2 lists mutation rates obtained by Methods II and III. There is apparently a correlation, in this case, between increase in culture size and decrease in mutation rate ($p = .03$). The average mutation rate is not significantly lower than that obtained by Method I, but it varies with the size of the culture being tested.

A selection pressure against h^+ would progressively lower the ratio of h^+ to h^- from that expected, and Method III would then give decreasing muta-

TABLE 2

Estimation of mutation rate from b^- to b^+ using Methods II and III.

	N	n	r	r_0	Mutation rate		Standard deviation*
	Number of cultures	b^- cells per culture $\times 10^8$			Method II $\times 10^{-8}$	Method III $\times 10^{-8}$	III $\times 10^{-8}$
1	12	.37	1.6	1	1.7	1.68	.81
2	24	.63	6.9	5	2.2	2.63	.85
3	12	.67	3.8	3	1.3	1.75	.46
4	7	.68	15.3	9	4.1	3.60	1.37
5	12	1.26	9.8	9	1.6	1.88	.56
6	9	1.49	15.1	9	1.8	1.64	.56
7	11	1.63	21.6	12	2.2	1.86	.53
8	6	2.28	14.6	12	1.4	1.41	.52
9	5	2.39	11.8	11	1.1	1.16	.61
10	7	11.79	99.0	95	1.2	1.25	.35
11	3	12.39	69.0	73	.9	1.01	.39
12	9	12.71	109.4	110	1.1	1.32	.30
					Weighted average	1.88×10^{-8}	
					Standard deviation	$.72 \times 10^{-8}$	

* Standard deviation after LEA and COULSON (1949).

tion rates with increasing culture size. To test the possibility of selection for or against h^+ cells in h^- cultures, one or more h^+ cells were introduced into some cultures at the time of inoculation. Ten different h^+ strains derived from spontaneously arising mutants and grown under the same conditions as were the h^- inocula were used in the various tests. The numbers of h^- and h^+ cells were determined after 10–15 generations and a calculation was made of the percent recovered of the number of h^+ expected if h^- and h^+ grow at the same rates. As shown by table 3, there is, on the average, no selection for or against h^+ cells.

The negative correlation between culture size and mutation rate could be

TABLE 3

Growth of b^+ in the presence of b^-.

	Total number of cultures	Cultures with b^+ added	t_1 Cells per culture		t_2 Cells per culture		Percent recovery of b^+
			b^- $\times 10^8$	b^+ $\times 10^0$	b^- $\times 10^8$	b^+ $\times 10^4$	
1	12	4	.22	.41	.68	1.37	110
2	12	6	.58	.69	2.29	3.38	124
3	24	12	.63	.69	.09	.08	81
4	12	3	1.77	.29	1.49	.31	126
5	11	10	2.27	2.40	1.25	1.74	132
6	23	18	5.10	1.57	2.39	1.02	138
7	12	9	6.54	1.39	12.39	2.39	91
8	11	10	12.00	2.40	4.26	.78	92
9	12	5	16.96	.54	11.79	.31	82
10	12	3	26.40	.29	12.71	.08	56
						Average	103

attributed to segregation if one assumes that h^+ is a dominant mutation occurring in one nucleus of a multinucleate cell. As in the case of selection against the mutant, the mutant frequency would not increase with culture size in the manner expected. Giemsa staining of h^+ and h^- cells in the logarithmic phase of growth reveals two to four chromatinic bodies per cell; ROBINOW (1945) and others have suggested that these bodies are analogous to cell nuclei, and thus the segregation hypothesis appears to have some morphological basis. Although h^+ is assumed to be dominant, the new mutant might require one or more cell divisions for the conversion of the h^- phenotype to h^+. As NEWCOMBE and HAWIRKO (1949) have pointed out, and as shown in figure 1, when phenotypic lag and segregation require the same period of time, no difference is expected in mutation rates calculated by Methods I and II. The data are in accord with the assumption that segregation extends over a period of about one generation. They would be equally consistent, however, with the hypothesis that segregation and the development of the h^+ phenotype occur simultaneously for a period of one or more generations but that segregation continues for at least one additional generation.

SPONTANEOUS MUTATION FROM h^+ TO h^-

The original penicillin selection method of DAVIS (1948) and LEDERBERG and ZINDER (1948) has been modified to increase the recovery of h^- mutants and to obtain an estimate of the percent of total mutants that are recovered. The optimal recovery of h^- consistent with the killing of the great majority of h^+ cells occurs with a penicillin concentration of 100 to 150 units per cc and an exposure of 8 to 10 hours at 37°C. No substance enhancing the selective killing action of penicillin under these experimental conditions has yet been found. The optimal concentration of h^+ cells is 5×10^6 per cc; when higher concentrations are used, the recovery of h^- is significantly decreased. For the most rapid killing, cultures to be tested should be in the logarithmic phase of growth. The penicillin method was made quantitative by adding a known number of h^-Lac^- cells to some of the cultures in each series and determining the percent recovery of the Lac^- cells after penicillin treatment. Assuming that a similar proportion of marked h^- cells and spontaneous h^- mutants is lost, it is possible to calculate the original number of mutants in each culture.

For example, 3 cc cultures of h^+Lac^+ cells were grown to the desired population size in medium containing histidine, washed, and resuspended in minimal medium. After assay of the h^+, penicillin was added to each culture to give a final concentration of 140 units per cc. At the same time, a certain number of h^-Lac^- cells were added to some or all of the cultures. After 10 hours at 37°C, the cultures were plated directly in minimal agar, each culture being divided between two plates. The plates were layered with minimal medium according to the technique of LEDERBERG and TATUM (1946). After 36 hours, the visible colonies, derived from h^+ cells not killed by penicillin,

TABLE 4

Comparison of recovery of h^- Lac^- and h^- Lac^+.

	h^+ per cc $\times 10^6$	No. of cultures	% recovery of h^-		S.E. of diff.	t	P
			Lac^+	Lac^-			
1	.2	12	29	32	15.3	.20	>.5
2	.6	10	42	49	6.5	1.08	.3
3	3.0	4	61	54	31.0	.22	>.5
4	3.6	12	26	50	6.9	3.48	.01

were marked, and a layer of minimal agar plus histidine was added. The additional colonies, presumably h^-, that appeared were streaked to Endo agar to determine the number of h^-Lac^- cells recovered. To avoid testing large numbers of h^-, the number of h^-Lac^- added per culture was approximately equal to the number of newly arisen h^- expected. The Lac^+ colonies were streaked from Endo to minimal agar to make certain that they were h^-; the percent that proved to be h^- varied from 50 to 100 percent, recovery being most efficient when the h^+ had been reduced to 50 or less per culture. The determined number of new h^- per culture was corrected by dividing by the percent survival of the h^-Lac^-.

To test the assumption that h^-Lac^- and h^-Lac^+ cells are killed at the same rate under similar conditions, experiments were set up in which equal numbers of (a) a mixture of spontaneously arising h^-Lac^+ or (b) h^-Lac^- cells were added to aliquots of minimal medium containing washed h^+ cells. The numbers of h^+ and both types of h^- cells were so adjusted that the contribution to the h^-Lac^+ class due to spontaneous mutation was negligible. The data from several experiments, summarized in table 4, show that in only one case was there a significant difference (at the 5 percent level) between the recovery of h^-Lac^- and h^-Lac^+ cells.

A number of h^- cells, derived from spontaneously arising mutants, were added to some of the h^+ cultures at the time of inoculation to determine whether there is selection against h^- mutants in predominantly h^+ cultures.

TABLE 5

Growth of h^- in the presence of h^+.

	N			t_1 Cells per culture		t_2 Cells per culture		% recov. h^-
	No. of cultures	Cultures with h^- added	% recov. from penicil.	h^+ $\times 10^3$	h^- $\times 10^{-1}$	h^+ $\times 10^6$	h^- $\times 10^2$	
1	34	5	61	2.24	1.59	.36	.05	20
2	34	9	75	3.77	3.08	.74	.34	56
3	17	2	18	4.52	1.26	2.92	.75	107
4	17	1	18	4.52	.61	9.03	.85	70
5	17	8	39	6.20	6.37	5.94	5.78	95
							Average	70

The h^- recovery, calculated as in the experiments on h^+ recovery, averaged 70 percent of that expected (table 5), indicating that selection would slightly lower the mutation rate as estimated by Methods II and III.

Table 6 lists the data obtained in experiments in which the penicillin method was used to estimate the frequency of h^- cells in h^+ cultures. The

TABLE 6

Estimation of mutation rate from b^+ to b^- using Methods II and III.

	Percent recovery of b^-	N No. of cultures	n b^+ per culture $\times 10^6$	r	r_0	Mutation rate Method II $\times 10^{-6}$	Mutation rate Method III $\times 10^{-6}$	Standard deviation III $\times 10^{-6}$
1	80	28	.36	1.6		1.27		
2	67	27	.38	1.4		1.09		
3	31	18	.62	4.5		1.55		
4	83	25	.74	2.3	1.2	.78	.90	.30
5	34	29	1.04	3.4		.73		
6	37	15	1.46	14.2	5.4	1.61	1.18	.34
7	38	11	1.85	23.7	7.9	2.16	1.23	.38
8	18	14	2.92	21.1	11.1	1.13	.98	.25
9	64	8	3.26	48.4	32.8	2.22	1.95	.54
10	42	9	5.94	41.9	23.8	1.07	.84	.23
11	39	11	10.98	133.3	120.5	1.54	1.63	.32
				Weighted average			1.17×10^{-6}	
				Standard deviation			$.36 \times 10^{-6}$	

experimentally observed P_0 cannot be used to determine the mutation rate by Method I because the "0" class includes some cultures which contained one or more h^- cells prior to treatment with penicillin. The probability that a culture containing r mutants before treatment will have no mutants after treatment is a function of r and S, the fraction of h^- surviving penicillin. Thus, for each culture

$$P_{0,c} = (1 - S)^r \qquad \text{IV}$$

This value, $P_{0,c}$, for each individual culture could then be applied as a correction factor if the frequency, P_r, of cultures having r mutants were known. Thus, the P_0 expected after penicillin treatment of a group of cultures is given by

$$P_0 = \sum_{r=0}^{r=n} (1 - S)^r (P_r)$$

LEA and COULSON (1949) have calculated P_r for various values of r from m, the average number of mutations per culture. An estimate of m can be obtained by Method II or, preferably, by Method III.

The values for the expected and observed P_0 agree closely, as shown in table 7. If an appreciable amount of phenotypic lag were present, the ob-

served P_0 would be higher than the expected P_0 which is based on the average or median number of mutants per culture. A statistical test has shown no correlation between culture size and the mutation rate obtained by Method III, indicating that mutant frequency increases with culture size in the manner expected in the absence of phenotypic lag. Adhering to the assumptions put forth to account for the discrepancies in mutation rates from h^- to h^+, it is necessary to postulate that if h^+ bacteria are multinucleate, a mutation to h^- cannot be detected in a cell simultaneously containing one or more h^+ nuclei. The absence of any detectable lag thus indicates that when h^- has segregated from h^+, the h^- phenotype appears rapidly.

ULTRAVIOLET-INDUCED MUTATION OF h^- TO h^+

Cultures to be irradiated were grown to 10^8–10^9 cells per cc and then washed twice with saline at room temperature over a period of an hour or more to stop bacterial multiplication. Ten-cc aliquots of the saline suspension were pipetted into Petri dishes which were rotated on a small turntable during irradiation. The ultraviolet source was a G.E. " Sterilamp " about 80 percent of whose ultraviolet radiation consists of the 2537 Å wave length. Nonreactivating yellow light from G.E. " Bugaway " bulbs was used throughout all experiments with irradiated cells. Since the bacterial concentration was not kept constant from experiment to experiment, dosage was measured in percent survival. After irradiation, the bacteria were transferred to centrifuge tubes and resuspended in sterile saline. A part of each treated culture was plated on minimal agar to determine the number of h^+ mutants present at zero time, and another part was diluted with medium containing histidine and allowed to divide one or more times. After growth, each culture was washed and assayed for h^- and h^+.

The technique of plating in medium with limited histidine enrichment in order to reveal induced mutants (DAVIS 1950) was unsuccessful; fewer h^+ colonies appeared on plates containing irradiated h^- cells than on control plates containing equal numbers of viable h^-. When h^+ cells were added to irradiated cultures before plating, there was 100 percent recovery of the

TABLE 7

Comparison of expected and observed P_0.

	Percent recovery of h^-	n h^+ per culture $\times 10^6$	m Mutations per cult.		P_0	
			From r	From r_0	Exp.	Obs.
1	80	.36	.66	....	.520	.500
2	67	.38	.60	....	.551	.704
3	31	.62	1.40	....	.490	.556
4	83	.74	.84	.96	.417	.440
5	34	1.04	1.10	....	.550	.553
6	37	1.46	3.40	2.50	.246	.200
7	38	1.85	5.80	3.25	.157	.273
8	18	2.92	4.80	4.15	.212	.285

added cells, showing that h^+ cells are not prevented from forming colonies. A possible reason for the failure of the limited enrichment method is the removal of the histidine present by the large numbers of "killed" irradiated cells which continue to metabolize but do not divide or give rise to viable h^+ cells. Although more tedious, the technique of growing irradiated cultures in liquid medium with optimal histidine also allows the cells to be tested at any interval after the initiation of growth.

Repeated tests have shown that h^- and h^+ cells are equally sensitive to irradiation when grown and irradiated under identical conditions. In order to determine whether there was selection for either type when h^- and h^+ were grown after irradiation, aliquots of a mixed culture were irradiated for various lengths of time. The irradiated cultures were grown in histidine-supplemented medium for 18 generations and then assayed for h^- and h^+. When approximately equal numbers of h^- and h^+ are irradiated there is no evidence of selection for either type. To test for selection under conditions more closely resembling those in irradiated h^- cultures, a small number of h^+Lac^- cells were added to irradiated cultures which were then grown for several generations. In six experiments in which h^+Lac^- cells were mixed with irradiated h^- in ratios of about 1 to 10^6 and grown for 15 generations, there was 89 percent recovery of the marked cells. Expected recovery was determined from cultures containing h^+Lac^- and unirradiated h^- cells. Selection is apparently not great enough to alter the major conclusions concerning induced reverse mutations.

When cultures are carefully washed before irradiation to halt cell division, "zero-point" h^+ mutations are not detected (fig. 2). Exceptions were found in the two earliest experiments of the series, but it has been impossible to repeat these observations in many trials. In all other experiments, there was a downward trend in h^+ frequency with increasing ultraviolet dose. Mutant frequency after growth (end-point mutation) was determined after one division and after five or more divisions of the irradiated cultures. To indicate the amount of variation in mutant frequency in different cultures before irradiation, the total h^+ frequency is plotted and thus includes both spontaneous and induced mutants. Apparently most induced mutants are expressed after one division but two or more divisions may be necessary for the expression of all h^+ mutants. In evaluating the data to determine the approximate duration of the lag in mutant expression, it is not necessary to consider the effects of irradiation-induced delay in division which is present in strain B/r (DEMEREC and LATARJET 1946; NEWCOMBE and SCOTT 1949). In both h^- and h^+ cultures, the surviving cells exhibit no greater delay than do unirradiated cells. This conclusion is based on determinations of cell increase in liquid cultures and on the surface of agar plates.

Because of the scatter between data from different experiments, it is difficult to determine whether the h^+ frequency increases linearly with dose. The log percent survival is inversely proportional to ultraviolet dose except for doses allowing over 50 percent survival. If mutant increase were to be plotted

against dose, the curve would show a slight flattening near the origin. Doses allowing less than 0.5 percent survival of h^- do not further increase the h^+ frequency, and the limited number of data for higher doses suggests that there may be a decrease in mutant frequency as has been observed for phage resistant mutants (DEMEREC and LATARJET 1946). NEWCOMBE and WHITEHEAD (1951) have found in *E. coli* that the frequency of color response mutants on mannitol-tetrazolium agar remains constant at doses over 500 ergs per sq. mm. A decline in mutant frequency with high doses of ultraviolet radiation occurs for the production of morphological and auxotrophic mutants in molds (HOLLAENDER and EMMONS 1941, 1946) and in Drosophila

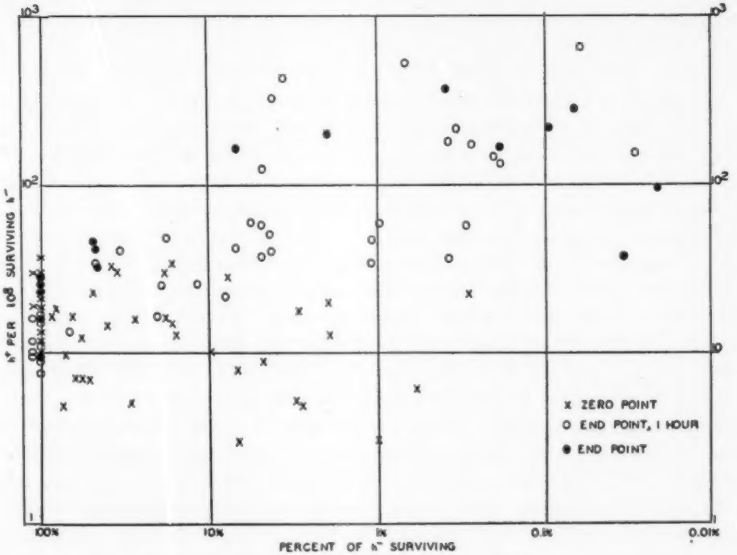

FIGURE 2.—Increase in the frequency of h^+ after ultraviolet irradiation.

(ALTENBURG, ALTENBURG, MEYER and MULLER 1950). This change in the relation of lethality to mutagenicity at high doses appears to be characteristic of ultraviolet treatment.

POST-IRRADIATION DEATH

In the course of the irradiation experiments, it was found that when irradiated non-growing cultures are plated immediately, the percent survival is many times greater than that observed if the cultures are assayed after a short period (30–50 minutes) of growth in liquid medium. The bacteria surviving this post-irradiation death divide at a normal rate unless the culture consists of h^- cells in a medium devoid of histidine, in which case the viable

count becomes stable. A careful study of the data suggests that the ultraviolet survivors immediately after irradiation consist of two types: "liquid resistants," which will divide at a normal rate in liquid medium, and "liquid sensitives," which die when placed in liquid medium but survive when plated. The ratio of percent survival immediately after ultraviolet to survival after growth in liquid is not constant, but increases with increasing dose. These observations are introduced here because post-irradiation death occurs between the measurement of "zero-point" and end-point mutants. Although it has been shown that h^- and h^+ cells do not differ in sensitivity to ultraviolet treatments that include post-irradiation growth in liquid, the possibility that there is a relationship between post-irradiation death and the production of mutations has not been ruled out.

ULTRAVIOLET-INDUCED MUTATION FROM h^+ TO h^-

The study of ultraviolet-induced mutation to h^- is limited to a low range of doses because the penicillin-selection method cannot be used effectively when the concentration of living plus ultraviolet-killed cells exceeds 5×10^6 per cc. The h^+ cells were washed and irradiated as were the h^- cells in the reversion experiments. Part of each irradiated suspension was grown in minimal medium containing histidine, part was tested immediately. After resuspension of the irradiated cells in minimal medium, penicillin and h^-Lac^- cells were added and the number of new h^- mutants determined as previously described in the section on spontaneous mutation. In addition to penicillin-killing, post-irradiation death, which affects both h^- and h^+ cells, occurs when bacteria are placed in liquid medium and penicillin after irradiation. Thus, it is impossible to determine the number of radiation survivors in these cultures either by assaying before the addition of penicillin or by assaying after the completion of post-irradiation death. A good approximation may be obtained by placing part of the irradiated suspension in liquid medium and assaying after one hour or by using a curve, obtained by pooling the data from several experiments, relating survival immediately after ultraviolet treatment to survival after growth in liquid medium. Penicillin has no effect on the post-irradiation death of h^- cells, and thus it may be expected that the death of h^+ is similarly unaffected.

Figure 3 shows that ultraviolet irradiation increases the frequency of mutation from h^+ to h^-. There is no detectable increase in mutant frequency when irradiated h^+ cultures are grown for one or more generations, so all mutations appear to be "zero-point" mutations. When evaluating these results, it should be borne in mind that penicillin does not kill all growing cells instantly. After one hour in penicillin, 90 percent of h^+ cells are incapable of dividing to form colonies even though they are freed of the drug. However, it is theoretically possible that an h^+ cell which has mutated to h^- might become phenotypically h^- during the early minutes of penicillin action, and later recover from the effects of penicillin. There is also the possibility that h^+ cells which have been washed thoroughly are essentially h^- in phenotype,

and remain so unless the h^+ gene(s) resumes its functions when the cells are replaced in culture medium. The term " zero-point " may be misleading and is, in any case, inaccurate, since a measurable length of time must elapse between mutagenic treatment and selection of the mutants.

The increase in h^- frequency relative to dose is very similar to the end-point h^+ increase in irradiated h^- cultures. Again, there appears to be a plateau or peak above which the mutant frequency does not rise although the

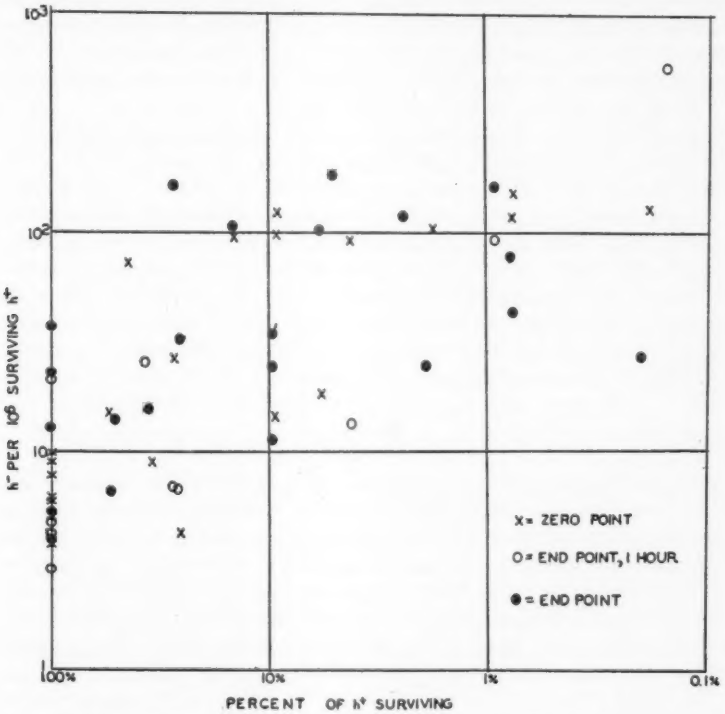

FIGURE 3.—Increase in the frequency of h^- after ultraviolet irradiation.

dose is increased. A considerable amount of variability is observed in the mutant frequencies at comparable doses; this was also observed by DEMEREC and LATARJET (1946).

COMPARISON OF INDEPENDENTLY ISOLATED MUTANTS

The growth rates of 22 spontaneous and induced h^+ mutants, each independently isolated, were compared turbidimetrically. In minimal medium, the growth curves of all strains appeared identical and were indistinguishable from the growth curve of the parent h^+ strain. The tests in which h^+ were

grown with h^- cells (table 3) provide further evidence of the similarity of the h^+ mutants. Nineteen h^+ strains were tested for the frequency of h^- mutants, which varied from 3.3 per 10^6 to 7.5 per 10^5 h^+. The similarity of mutant frequencies strongly suggests that the rate of mutation from h^+ to h^- is almost identical in all the tested strains. DAVIS (1950) found growth curves differing from wild-type for the majority of back mutants from several different auxotrophs. Many of these prototrophs were "partial back mutants," requiring a growth factor for optimal growth. If h^- strain A-11 gave rise to such mutants, these slow-growing prototrophs should have been detected, for the h^+ mutants to be tested were often isolated from minimal agar several days after plating. The similarity of the h^+ strains suggests that h^- strain A-11 reverts to h^+ by a single mutation.

The growth rates of 28 h^- strains, both spontaneous and induced, were studied in medium containing histidine. Although the curves varied somewhat, the final level of growth was the same for all mutants. A slight difference in growth rates among various strains is reflected in the results of selection tests (table 5). The frequency of h^+ mutants varied from 0 per 10^9 to 1 per 10^5, indicating that the rates of mutation to h^+ and/or selection of h^+ mutants differ significantly in the various h^- strains. At least three different h^- types appear to be present on the basis of reversion frequency; apparently strain A-11 carries only one of several possible histidine-requiring mutations, which may represent allelic mutations or mutations of different genes.

DISCUSSION

A comparison of spontaneous mutation rates with ultraviolet-induced mutation frequencies indicates that the h^+ and h^- characters retain their relative mutability when subjected to ultraviolet radiation. However, while the rate of spontaneous mutation to phage T1 resistance in strain B/r is similar to that of h^- and h^+ in strain 15, the frequency of phage-resistant mutants was increased to a far greater extent by ultraviolet treatment. One cannot be certain whether this inconsistency is a reflection of a difference in the particular genetic system involved or of differences in the entire genetic constitutions of the two strains. The majority of the data now available suggest that, in *E. coli,* the relative spontaneous mutability of a character is an indication of its relative mutability when treated with ultraviolet radiation.

The relation between dose and the production of h^- and h^+ mutations differs considerably from that found for T1 resistance (DEMEREC and LATARJET 1946). If the h^+ data are corrected for mutations present before irradiation, the curve of mutant increase shows a steady rise to a maximum, in contrast to the sharp initial rise in the end-point mutant curve for T1 resistance. This initial rise has not been observed in any other studies of induced mutation and may be unique for phage resistant mutants or associated with the conditions of the experiment (LEDERBERG 1949).

The assumption has been made that the bacteria contained in this study

contained about two nuclei, and that one h^+ nucleus is dominant to one or more h^- nuclei. This conclusion is based primarily on the discrepancies in mutation rate calculations for the spontaneous mutation of h^- to h^+. If h^+ is dominant, segregation cannot be detected in a study of spontaneous mutation from h^+ to h^-, but should be observed as a delay in the appearance of induced h^- mutants. Some of the possible causes for the failure to detect any lag have been discussed above, and one should also consider the possibility that ultraviolet radiation produces a considerable amount of nuclear killing, so that many of the cells surviving radiation may be effectively mononucleate. Since the duration of the expected segregation lag is only about one division, the failure to detect it with present methods cannot be considered conclusive evidence of its absence.

Phenotypic lag, unless obscured by nuclear segregation, is apparently not present in the spontaneous mutation of h^- to h^+ or h^+ to h^-. However, a "priming" (DAVIS 1950) period of about one division is required for the expression of induced h^+ mutations. Since h^+ is apparently dominant, lag cannot be attributed to the necessity for nuclear segregation. The lag in h^+ expression is not one of time alone, since induced h^+ mutations never appear if the cells are plated in minimal medium without previous growth in histidine. This suggests that for h^+ expression, there must be the production of a new material, involving cellular growth, rather than the decay of some product(s) of the h^- character. In the case of spontaneous mutation, phenotypic lag may be of shorter duration, or may be obscured by segregation.

SUMMARY

1. A method has been developed for the quantitative recovery of growth-factor-requiring mutants from their parental cultures.

2. The rate of spontaneous mutation from h^+ to h^- in the prototrophic strain studied is 1.2×10^{-6} per bacterium per division cycle, and the reversion rate of a derived h^- strain is 2.9×10^{-8} per bacterium per division cycle.

3. Equivalent doses of ultraviolet radiation increased both mutation rates by approximately the same factor. The mutant frequency increased with dose, reaching a maximum of about $100 \times$ the original frequency at 0.5 to 1.0 percent survival.

4. No phenotypic lag was detected for the expression of spontaneous h^- and h^+ mutations. After irradiation, h^+ mutations were not expressed unless the culture was placed for about one hour in a culture medium containing histidine, but h^- mutations did not require this treatment for expression.

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ON THE INTERPRETATION OF THE DOSE-FREQUENCY CURVE IN RADIOGENETICS *

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IN an article under the same title by OPATOWSKI (1950) and in another by OPATOWSKI and CHRISTIANSEN (1950) doubt has been raised whether a linear relationship between mutation frequency and irradiated dose, at low dosage, can be considered as proof that a mutation is induced by one event. The conclusion reached in these papers is that under the opposite assumption, *i.e.*, that a mutation needs a large number of primary events, it is quite possible to get a frequency-dose curve which is essentially linear in the range in which measurements have been made (SPENCER and STERN 1948). Since this conclusion seems at first sight rather strange, it was considered worth while to make a more thorough analysis of the problem.

The purpose of this paper is to investigate in several cases whether or not the theoretical mutation frequency curve can be distinguished from a single event-type curve, which is essentially a straight line in the lower range of the curve. The question how well the experimental data justify a straight line fit, is of an entirely different nature and will not be considered here. Since the experimental data cover a mutation frequency range from about 0.001 to 0.1, the theoretical curves will be investigated in this same range.

Our calculations will be based on the following model: A certain mutation is a certain chemical change in a particular gene, effected by one or more ionizations or excitations in that gene. An ionization or excitation will henceforth be called an "event." We suppose that the events are independent of each other. Let n be the number of events which have to occur in order to produce the mutation, and let N be the total number of events which possibly can take place in the gene, so that we have $n \leqq N$. Since the gene may be a big molecule, N may be very large. However, we do not want to include in N any possible events which cannot contribute to the mutation under consideration. Therefore, even if the gene is a big molecule the number N is not necessarily large (it may be even 1). We shall distinguish between two cases: 1) $N \gg 1$, and 2) N not $\gg 1$. In case 1) we let N approach infinity in order to simplify the calculations. In both cases we distinguish further between the two possibilities: a) all events have the same probability of occurrence; b) the events may have different probabilities. In each case the mutation frequency is the probability that out of N possible events at least n events actually occur. The model described above does not cover all possible cases, but it covers a range sufficiently wide to be of interest.

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[1] PUBLIC HEALTH SERVICE RESEARCH FELLOW OF THE NATIONAL CANCER INSTITUTE.

The existence of spontaneous mutations is no complication in the present treatment, since, following OPATOWSKI, we can consider these spontaneous mutations as being caused by a fictitious radiation of dose S. If we denote the dosage of the actual radiation by D, the total effective dose is D + S.

After application of this effective dose the probability that the i^{th} event has not taken place is $q_i = \exp\left[-c_i(D+S)\right]$, in which c_i is a constant. It follows that the probability p_i that the i^{th} event has taken place is

$$p_i = 1 - q_i = 1 - \exp\left[-c_i(D+S)\right].$$

For convenience we introduce the following dimensionless quantities

$$c = \sum_{i=1}^{N} c_i, \quad f_i = \frac{c_i}{c},$$

and:

$$x = c(D+S) \tag{1}$$

so that we have:

$$\sum_{i=1}^{N} f_i = 1 \tag{2}$$

$$q_i = \exp\left[-f_i x\right] \tag{3}$$

$$p_i = 1 - \exp\left[-f_i x\right] \tag{4}$$

We now proceed to discuss the four different cases 1a, 1b, 2a and 2b separately.

Case 1a: ($N \to \infty$, f_i is the same for all i).

From (2) follows $f_i = 1/N$ and substitution into (4) gives $p_i = p = 1 - \exp\left[-\frac{x}{N}\right]$. The average number of events is

$$\lim_{N\to\infty} Np = \lim_{N\to\infty} N\left\{1 - \exp\left[-\frac{x}{N}\right]\right\} = x,$$

which can be seen most easily after expanding the exponential in a power series. Now in our case, since $N \to \infty$, the probability of exactly m events is given by Poisson's formula:

$$P_m = \frac{1}{m!}\, e^{-x} x^m \tag{5}$$

If at least n events are necessary for the mutation, then the probability M_n of the mutation is $M_n = 1 - \sum_{m=0}^{n-1} P_m$, or, after substitution of (5):

$$M_n = 1 - \sum_{m=0}^{n-1} \frac{1}{m!}\, e^{-x} x^m \tag{6}$$

which also can be written in the form:

$$M_n = \frac{1}{(n-1)!} \int_0^x y^{n-1} e^{-y} dy \tag{7}$$

That eqs. (6) and (7) are equivalent can be seen by integrating (7) by parts n times.

M_n can be plotted as a function of x for various values of n. For small n formula (6) is convenient, for large n formula (7) can be used to investigate the shape of the mutation frequency curve in the limit of $n \to \infty$. In taking this limit we may replace $n-1$ in (7) by n:

$$M_\infty = \lim_{n \to \infty} \frac{1}{n!} \int_0^x y^n e^{-y} dy \tag{8}$$

If n is large compared with 1 we can approximate the integrand in (8) by a Gaussian function, *i.e.*, a function of the form $A \exp[-B(y-C)^2]$, with A, B and C positive constants, and choose these constants such as to let the two curves have the same maximum and the same curvature at the maximum. The maximum of $y^n e^{-y}$ lies at $y = n$, and the value at the maximum is, of course, $n^n e^{-n}$, so $C = n$ and $A = n^n e^{-n}$. The second derivative of $y^n e^{-y}$ at $y = n$ is $-n^{n-1} e^{-n}$; the second derivative of $n^n e^{-n} \exp[-B(y-n)^2]$ at $y = n$ is $-2B n^n e^{-n}$. Equating the second derivatives of the two functions gives $B = \frac{1}{2}n$. We have, finally:

$$y^n e^{-y} \cong n^n e^{-n} \exp\left[-\frac{1}{2n}(y-n)^2\right], \quad (n \gg 1) \tag{9}$$

For $n \gg 1$ this function has a sharp peak at $y = n$, and if we take in (8) the lower limit of the integral $-\infty$ instead of 0, the error we make approaches zero as n approaches infinity. Thus we have:

$$M_\infty = \lim_{n \to \infty} \frac{1}{n!}\, n^n e^{-n} \int_{-\infty}^x \exp\left[-\frac{1}{2n}(y-n)^2\right] dy.$$

Using Stirling's approximation:

$$n! \cong n^n e^{-n} \sqrt{2\pi n}, \quad (n \gg 1) \tag{10}$$

and denoting by $H(x)$ the function:

$$H(x) = \frac{2}{\sqrt{\pi}} \int_0^x e^{-\xi^2} d\xi$$

we can put M_∞ in the form:

$$M_\infty = \lim_{n\to\infty} \tfrac{1}{2}\left\{1-H\left(\frac{n-x}{\sqrt{2n}}\right)\right\} \tag{11}$$

It has been stated before that we take the mutation frequency of spontaneous mutations as $M = 0.001$. Let this value be assumed in $x = x_0$, then x_0 corresponds to $D = 0$. If we substitute $M = 0.001$ and $x = x_0$ into (11) we can solve for the argument of the function H. Using the Tables of the W. P. A. PROJECT FOR THE CITY OF NEW YORK (1941) we obtain: $\lim_{n\to\infty} \frac{n-x_0}{\sqrt{2n}} = 2.185$. We now put $\lim_{n\to\infty} \frac{x-x_0}{\sqrt{2n}} = z$, so that $\lim_{n\to\infty} \frac{n-x}{\sqrt{2n}} = 2.185-z$. It is important to note that z is a variable proportional to D. In terms of the new variable (11) becomes:

$$M_\infty = \tfrac{1}{2}\{1-H(2.185-z)\}, \quad (z \geqq 0) \tag{12}$$

By plotting M_∞ against z one obtains the shape of the mutation frequency curve, with $n \to \infty$, for values of $M \geqq 0.001$.

The results so far obtained have been plotted in figure 1 with solid lines. Curve *a* is M_1, curve *b* is M_2, both curves calculated from (6): curve *c* is M_∞, calculated from (12). The curves for $n = 3$, 4, etc., not drawn in the figure, lie between curves *b* and *c*. For the sake of comparison the curves have been shifted and compressed in horizontal direction in such a way as to make them intersect at $M = 0.001$ and $M = 0.1$.

It is easily seen from figure 1 that the only curve which is essentially linear in the range considered is curve *a, i.e.,* the curve based on a single event model. The curve for $n = 2$ deviates conspicuously from a straight line, and the deviation becomes greater with increasing n.

Case 1b: ($N \to \infty$, f_i arbitrary).

As in case 1a we denote by P_m the probability of exactly m events, and by M_n the probability of at least n events, so that we still have $M_n = 1 - \sum_{m=0}^{n-1} P_m$. However, because of the arbitrariness of the f_i, P_m is not necessarily given by Poisson's formula (5), and, therefore, the expression (6) for M_n may not hold in this case. We shall investigate under which conditions the equations (5) and (6) will still be valid.

The probability P_0 of no events at all is the product of the probabilities q_i, given by (3):

$$P_0 = \lim_{N\to\infty} \prod_{i=1}^{N} q_i = \lim_{N\to\infty} \prod_{i=1}^{N} \exp[-f_i x] = e^{-x}$$

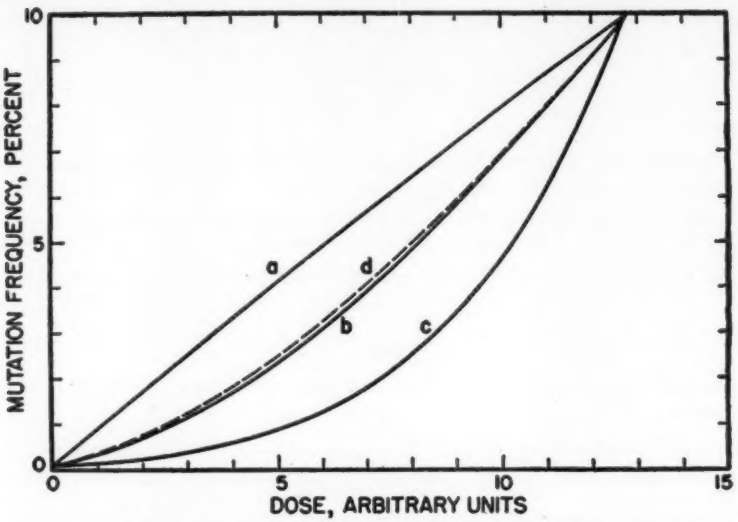

FIGURE 1.—Mutation frequency M as a function of the radiation dose D, in the frequency range $0.001 \leqq M \leqq 0.1$, for several values of n and N. Curve *a*: n = 1, N arbitrary; curve *b*: n = 2, N = ∞; curve *c*: n = ∞, N = ∞; curve *d*: n = 2, N = 2. All other values of n and N give curves lying between the curves *d* and *c*.

The single event curve *a* is compared with the multiple event curves *b*, *c* and *d*. The single event curve is approximately a straight line, whereas the multiple event curves are obviously curved.

in view of (2). This is the same expression as given by Poisson's formula (5), with m = 0, so that the mutation frequency for n = 1, $M_1 = 1 - e^{-x}$, is the same as in case 1a. This result is independent of special assumptions concerning the f_i.

The probability of exactly one event is

$$P_1 = \lim_{N\to\infty} \sum_{i=1}^{N} p_i \prod_{j\neq i} q_j = \lim_{N\to\infty} \sum_{i=1}^{N} (1-\exp[-f_i x])\exp[(-1+f_i)x],$$

which can be brought into the form:

$$P_1 = e^{-x} \lim_{N\to\infty} \sum_{i=1}^{N} (e^{f_i x} - 1) \tag{13}$$

After expanding the exponentials under the summation sign, (13) can be written

$$P_1 = e^{-x} \lim_{N\to\infty} \sum_{i=1}^{N} (f_i x + \tfrac{1}{2} f_i^2 x^2 + \ldots) \tag{14}$$

It is impossible to examine the shape of P_1 unless we make some assumption about the f_i. The physically most plausible assumption is that, although the f_i may be quite different from each other, none of them will be very much larger than any of the others. In terms of our physical model this means that none of the events which can take place in the gene occurs with a much greater probability than any of the other events. Under this assumption, and in view of (2), it is clear that $\lim f_i = 0$ for each i, in such a way that

$$\lim_{N\to\infty} \sum_{i=1}^{N} f_i^m = 0 \text{ for } m \geq 2.$$

In (14) we keep only the terms of first order: $P_i = e^{-x} \lim_{N\to\infty} \sum_{i=1}^{N} f_i x = xe^{-x}$ by virtue of (2). This is Poisson's formula (5) with $m = 1$. Thus we get for M_2 in case 1b the same expression as in case 1a, *i.e.*, (6) with $n = 2$. By the same type of argument it can be shown that, in general, P_m is given by (5), and, therefore, M_n is given by (6) just as though all the f_i were equal. Therefore, under the assumption made concerning the f_i, we reach the same conclusion as in case 1a.

If we do not assume that none of the f_i is extraordinarily large compared to any of the others, we must allow a possibility that, for instance, one of the f_i is large compared to the sum of all the other f_i. It can be shown that in this case an n-event model produces an $(n-1)$-event type curve, *i.e.*, a curve given by (6) after replacing n by $n-1$. Physically the reason for this is clear: if the radiation dose is sufficient to produce with appreciable probability one of the events with small f_i, the one event which is comparatively so much more probable will then have been produced simultaneously with almost certainty, so that the existence of this event has no influence on the shape of the mutation frequency curve. Similarly, if there are k events, each of which is much more probable than the other $N-k$ events together, the number characterizing the type of mutation frequency curve is reduced by k. In particular, if $n = k+1$, *i.e.*, the minimum number of events necessary to cause a mutation is one more than the number of exceptionally probable events, the mutation frequency curve will have the shape of a one event curve.

Since there does not seem to be direct evidence against the above mentioned possibilities, we cannot quite exclude them. However, these exceptional possibilities seem physically rather unreasonable and unlikely, and we shall not consider them any further.

Case 2a: $\left(N \text{ not} \gg 1,\ f_i = \frac{1}{N} = \text{same for all } i\right)$.

The difference between this case and the preceding cases again lies in the expression for the probability P_m of exactly m events. As in case 1a, the probabilities of occurrence and of not occurrence of the i^{th} event are

$p = 1 - \exp\left[-\frac{x}{N}\right]$ and $q = \exp\left[-\frac{x}{N}\right]$ respectively, independent of i. From this we derive:

$$P_m = \binom{N}{m} p^m q^{N-m} = \binom{N}{m} \left(1 - \exp\left[-\frac{x}{N}\right]\right)^m \left(\exp\left[-\frac{x}{N}\right]\right)^{N-m},$$

or

$$P_m = \binom{N}{m} e^{-x} (e^{\frac{x}{N}} - 1)^m \tag{15}$$

For $m = 0$ we still have $P_m = e^{-x}$, just as in the case $N \to \infty$ so that M_1 is still given by (6) with $n = 1$, representing a one event type curve. For P_1 we find from (15): $P_1 = e^{-x} N(e^{x/N} - 1)$, and after expanding the exponential we get:

$$M_2 = 1 - P_0 - P_1 = 1 - e^{-x} - xe^{-x}\left(1 + \frac{x}{2N} + \frac{x^2}{6N^2} + \ldots\right) \tag{16}$$

The bigger N is, the more M_2 approaches $1 - e^{-x} - xe^{-x}$, which is the expression for M_2 in the case 1a or b, and which was already shown to be non-linear in the pertinent range. In the worst case $N = 2$, in which we can write M_2 as:

$$M_2 = (1 - e^{-\frac{x}{2}})^2, \quad (N = 2) \tag{17}$$

This curve has been plotted in figure 1 as curve *d* (dotted line). Again the horizontal scale has been adjusted such as to facilitate comparison with the other curves. It is seen that curve *d* is very close to curve *b*, and can be distinguished very well from the one event curve *a*. Since the curves for $n = 2$ and $N > 2$ all lie between curves *b* and *d*, our conclusion holds for $n = 2$ and any N.

If one investigates the curves for $n = 3$ and various $N \geqq 3$, it turns out that none of these curves is straighter than $(1 - e^{-x/3})^3$. Since the latter is curved more than $(1 - e^{-x/2})^2$, is follows that all curves for $n = 3$ and any N can be well distinguished from the one event curve M_1. The same conclusion holds for higher values of n.

Case 2b: (N not $\gg$ 1, f_i arbitrary)

Again we have M_1 given by (6) with $n = 1$. M_2 is equal to

$$M_2 = 1 - e^{-x} - xe^{-x}\left(1 + \tfrac{1}{2}x \sum_{i=1}^{n} f_i^2 + \ldots\right).$$

Under the same assumptions as made in case 1b, *i.e.*, that none of the f_i is very large compared with the other f_i, each f_i is of the order of magnitude $\frac{1}{N}$, and therefore $\sum_{i=1}^{n} f_i^2$ is of the order $N \cdot \frac{1}{N^2} = \frac{1}{N}$. We write $\sum_{i=1}^{n} f_i^2 = \frac{a}{N}$, in which *a* is a constant close to 1. Substitution into M_2 gives:

$$M_2 = 1 - e^{-x} - xe^{-x}\left(1 + a\frac{x}{2N} + \ldots\right) \tag{18}$$

Since the constant a is close to 1, and appears in front of a term which itself is a correction term, M_2 given by (18) is very close to M_2 given by (16), which was already shown to be appreciably curved.

It can be shown that also for higher values of n the expressions for M_n are well approximated by the corresponding expressions in case 2a, all of which yielded curves which could be well distinguished from the one event curve.

CONCLUSION AND COMPARISON WITH OPATOWSKI'S RESULTS

We conclude from the foregoing that, if our physical model is correct, a linear relationship between mutation frequency and radiation dose is a strong indication that a mutation is caused by a single event.

The question arises why the conclusions of this paper are so very different from those reached in the papers by OPATOWSKI and OPATOWSKI and CHRISTIANSEN. In order to answer this question we shall examine the equation derived in OPATOWSKI's paper, which is equation (3) in that, paper, and which will be denoted here by (O3):

$$P(D) = C\int_{-s}^{\infty} \exp\left[-h^3(x-M)^3\right] dx \qquad (O3)$$

In this equation D and S have the same meaning as in the present paper; P is the mutation frequency (which was called M throughout this paper); h and M are constants, the values of which can be chosen arbitrarily and independently; and C is determined by the normalization $P(\infty) = 1$.

The assumptions underlying OPATOWSKI's derivation of (O3) are equivalent with the assumptions of case 1a in the present paper. Therefore, we should be able to compare (O3) with (7), with the understanding that $n \gg 1$. Using the Gaussian approximation (9) and Stirling's approximation (10), we can write (7) as:

$$M_n \cong \frac{1}{\sqrt{2\pi(n-1)}}\int_0^x \exp\left[-\frac{1}{2(n-1)}(y-n+1)^2\right]dy, \quad (n \gg 1) \qquad (19)$$

The integration limits in (19) are $y = 0$ and $y = x$. In order to compare (19) with (O3) we have to introduce a new integration variable ξ:

$$\xi = \frac{y}{c} - S \qquad (20)$$

such that the integration limits become $-S$ and D. Indeed, when $y = 0$, $\xi = -S$, and when $y = x$, $\xi = x/c - S = D$, according to eq. (1). In order to get (19) entirely in the form (O3) we replace the two constants n and c by two other constants M and h by making the substitutions:

$$n = 1 + 2h^3(M+S)^3 \qquad (21)$$

$$c = 2h^3(M+S) \qquad (22)$$

After substituting (20), (21) and (22) into (19) we get:

$$M_n \cong \frac{h}{\sqrt{\pi}} \int_{-s}^{D} \exp[-h^2(\xi - M)^2]\, d\xi \qquad (23)$$

which is identical with (O3). The only non-essential differences between the two equations are that the integration variable is called x in (O3) and ξ in (23), and that the normalization constant C which is left undetermined in (O3) is written down explicitly in (23). The value of C is only equal to $h/\sqrt{\pi}$, though, if $n \gg 1$, but if this condition does not hold the derivations of both (O3) and (23) would be invalid anyway.

So far there seems to be complete agreement. The difficulties arise only through a particular choice of values for the as yet undetermined constants in (O3).

From eq. (21) follows that any choice of values for h, M and S determines the value of n. Since the whole derivation of (O3), and of (23) was based on the assumption of $n \gg 1$ we only have freedom to choose values for h, M and S as long as they are not in contradiction with the requirement $n \gg 1$. OPATOWSKI tries to make (O3) fit a straight line, and succeeds with the following values of the constants: $h = 2.5 \cdot 10^{-5}$ (roentgen)$^{-1}$, $M + S = 6642$ roentgen. After substitution of these values into (21) we obtain: $n = 1.055$, which certainly violates the requirement $n \gg 1$. Hence OPATOWSKI's conclusion, *i.e.*, that a many-event model can give a straight mutation frequency curve, is invalid. On the contrary, in trying to make (O3) fit a straight line it turns out that n is essentially 1, which is another proof that a straight line indicates a single event mechanism. That n does not turn out to be exactly 1 is a result of the fact that the Gaussian approximation (9) is rather poor for such a small n.

In a recent article by BOAG (1951), in which he points out the fallacy in OPATOWSKI's paper in a slightly different way, a sketch is made of the integrand $\exp[-h^2(x - M)^2]$ in (O3), using the previously mentioned values of the constants.* Due to the smallness of h this function falls off so slowly on both sides of the maximum, that at $x = -S$ the function still has practically maximum value. Thus it becomes clear why this function comes closest to an aproximation of a simple exponential e^{-x}, indicative for a single event mechanism.

SUMMARY

The question whether a linear relationship between mutation frequency and radiation dose is a strong indication that a mutation is caused by a single event has been investigated in several cases, and answered in the affirmative. The results of the calculations, supporting this conclusion, are shown in the

* The author is indebted to DR. BOAG for being able to see his manuscript before publication.

graphs of figure 1, in which the curves for multiple-event models are compared with the curve for a single-event model.

An exception has to be made for the possibility that some events may occur with much higher probability than any of the others, in which case a multiple-event model could give rise to a single-event type curve. However, this case is physically unlikely.

Finally it is shown that OPATOWSKI's curve, which is supposed to represent a many-event mechanism, actually represents a one-event mechanism, so that his conclusion that a many-event theory can give rise to a linear curve is unjustified.

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REDUCTION IN FACET NUMBER IN BAR-EYED DROSOPHILA BY X-RAYS

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THIS paper deals with a quantitative well-controlled response to X-rays: reduction of eye facet number in Bar-eyed flies. The experimental results will be used to discuss the problem of the differentiation of the eye facets in general and the nature of the mode of action of the Bar mutants.

MATERIALS AND METHODS

Larvae of an inbred Bar-eyed race of *Drosophila melanogaster* were treated with X-radiation of dosages varying from 115 to 1840 r units. The strain used in these experiments was derived from a spontaneous reversion to Bar (B) from Bar-Infrabar (BB^i). The latter stock has been kept with continuous inbreeding since its origin in 1931 from a mating between a heterozygous Bar by Infrabar female by an Infrabar male. Eggs were collected from 5–10-day-old adult flies on cardboard milk bottle caps on each of which a blob of cornmeal, molasses, brewer's yeast, agar food was centered. Just before using, these blobs were saturated with the following solutions: solution (1) 50% distilled water, 50% Fleischman's compressed yeast; solution (2) 88% distilled water, 10% molasses, 2% glacial acetic acid. Inverted one-half-pint urine analysis bottles served as containers for the flies while collecting eggs. The temperature during the egg laying was 24.0 ± 1.0° C and the duration of the egg laying periods varied from 1½ to 3 hours. The caps containing the eggs were put in 100 × 15 mm Petri dishes and placed at 28.0 ± 0.3°C; at which temperature the eggs and larvae continued their development until irradiated. During the time when irradiation was being done the entire set of Petri dishes including the controls were taken to the room where the X-ray machine was located. While in transit the Petri dishes were packed in a heavy cardboard carton which was wrapped in cotton batting, so that the temperature could not deviate far from 28°C. Immediately after the completion of the treatments the treated larvae and the controls were returned to the 28°C incubator where subsequent development took place. The controls were picked at random throughout the experimental series and were treated exactly the same as all others except that they were not irradiated. The mid point of the egg laying period was used as the zero point in computing egg or larval age. When the adult flies emerged they were etherized and preserved in 95 percent alcohol until the facet counts could be made. In all cases the right eye only was counted. The facet counting was done by the senior author.

The irradiation factors were as follows: 100 KV, no filter, 10 mA, target distance 9.75 cm, dosage rate (including scatter) 1380 r/min in the middle of the blob, with the extremes 5–10 percent off in either direction.

It was noted that rayed larvae pupate later than the controls, in agreement with the findings by HUSSEY, THOMPSON, TENNANT and CAMPBELL (1932). No attempt was made to measure this effect. The X-ray treatment did not produce noticeable morphological effects in structures other than the eyes. Antennae, bristles, wings, halters, and legs appeared to be normal in the X-ray treated series.

RESULTS

Preliminary tests

Preliminary tests were carried out to determine the effective dosage range and to find the general location of a radiosensitive period. The results are summarized in table 1. It was clearly demonstrated that X-rays reduce mean facet number and that the period of response is largely confined to a definite radiosensitive period. Thus larvae that are treated before they are 45 hours of age show no reduction. Likewise, larvae that are 73–75 hours or older show no effect except when treated with 1380 and 1840 r. At these dosages there is a slight but significant reduction but the response is obviously not so great as with larvae X-rayed when younger. An abstract of the preliminary results has been published elsewhere (LUCE, CHASE and QUASTLER 1948).

Lethal effects

The preliminary tests indicated that the egg and early larval stages are especially sensitive to X-rays in that only small numbers survived and produced imagoes, agreeing with the results of PACKARD (1935). At all dosages above 115 r, so few larvae survived the treatments in these early stages and such small numbers emerged as adults that it was impossible to obtain even an estimate of the effect on the eyes. With the 115 r treatments sufficient numbers of adults emerged to obtain good averages for the facet numbers of the adult eyes. Here it was found in both the 5–23 and the 27–44-hr. age groups that the X-rays *increased* rather than decreased facet number.

The lethal effect at other than the early stages was much less pronounced. With dosages of 920 r and less applied at 40 hours of egg larval life and at all later stages up to 86 hours, the number of adults that emerged as imagoes from the treated larvae was no different from the number emerging in the case of the controls. With dosages of 1380 and 1840 r, however, there was considerable lethality at all larval stages. The adults which emerged after treatment with these dosages were so weak, in many cases, that they became entangled in the food film and had to be removed with needles for preservation. In some other cases the treated larvae produced completely formed imagoes that were unable to emerge from the pupal cases. These were dissected out with needles. In a very few instances at 1840 r the puparia which were dissected contained incompletely developed imagoes. The head region

TABLE 1

Preliminary Tests.

Dose in r units	Age at time of irradiation	Females			Males		
		No.	Mean facet number	Stan. error	No.	Mean facet number	Stan. error
Control	60–82	76	41.5	0.5	82	44.7	0.5
460	60–67	78	37.0	0.5	58	38.9	0.7
460	73–82	35	43.2	0.6	47	46.8	0.8
920	60–69	73	31.0	0.6	65	29.0	0.8
920	70–82	34	43.2	1.1	29	45.7	1.0
1840	60–67	40	19.0	1.4	29	10.3	1.1
1840	70	16	28.5	1.5	14	19.8	1.9
1840	74–82	22	36.0	1.2	13	40.8	1.9
Control	8–86	300	42.5	0.3	265	44.3	0.3
115	5–23	43	44.8	0.6	44	48.1	0.7
115	27–44	96	44.8	0.5	81	46.9	0.6
115	47–53	69	41.4	0.6	64	41.2	0.4
115	57–67	37	41.2	0.7	35	41.4	0.6
115	77–81	16	42.5	1.2	26	45.9	0.8
460	38–44	38	43.5	0.6	46	44.9	0.6
460	47	23	40.0	0.7	20	40.9	0.6
460	50–53	62	38.2	0.6			
460	50				38	38.8	0.8
460	53				8	35.4	1.0
460	57–65	47	36.3	0.6	45	36.3	0.6
460	69	48	36.3	0.5	50	39.0	0.7
460	73–81	57	43.4	0.7	41	43.6	1.0
920	34–44	38	43.3	0.6	24	44.0	0.9
920	47–50	16	40.4	0.9			
920	47				8	38.8	1.0
920	50				13	34.0	1.2
920	53	18	35.2	1.0	13	33.0	1.5
920	57–65	102	29.0	0.6	86	26.5	0.9
920	69	12	31.5	1.4	5	26.6	3.4
920	77–81	42	41.1	0.9	46	42.4	1.0
1380	44	6	43.5	1.6	6	42.8	0.9
1380	47–53	11	36.1	1.7	13	37.4	2.1
1380	57–65	15	28.8	3.1	20	25.9	3.2
1380	69	51	26.6	1.0	34	26.4	1.5
1380	77–86	55	38.7	1.1	42	41.1	1.3

was specially defective, usually consisting of a simple sac covered with an outer unpigmented integument with little or no signs of differentiation into hairs, bristles, antennae, and ommatidia.

The radiosensitive period

Table 2 and figure 1 record the results of tests to find more exactly the limits of the radiosensitive period for facet reduction. The dose used was 920 r which, in preliminary test, had given good facet reduction and little mortality. In both sexes, the radiosensitive period begins near 50 hours. Maximum sensitivity is reached between the 57th and 67th hour. At 77–80 hours nearly but not quite all sensitivity disappears.

TABLE 2

Test for most sensitive period, 920 r raised at 28 degrees C.

Age of larvae when irradiated	Females			Males		
	No.	Mean facet number	Stan. error	No.	Mean facet number	Stan. error
Control	76	42.7	0.5	71	45.9	0.6
40	46	42.5	0.5	40	45.5	0.7
42	23	41.3	0.6	21	45.4	0.8
44	49	41.5	0.6	50	41.5	0.7
46	50	40.5	0.6	35	40.7	0.6
48	50	41.2	0.6	41	41.8	0.6
50	40	39.7	0.9	48	36.5	0.9
52	34	38.4	1.0	41	35.6	0.9
55	27	39.1	0.8	33	34.2	1.0
57	27	34.6	0.9	30	30.2	1.4
59	25	26.8	0.8	24	24.4	0.8
61	26	31.4	1.1	40	26.4	0.9
63	28	28.6	1.0	33	25.8	1.2
65	39	27.1	0.7	37	26.2	0.7
67	35	28.6	1.0	25	29.4	1.3
70	45	33.4	0.7	50	33.4	1.0
73	60	34.6	0.6	59	37.2	0.8
77	35	34.5	1.1	40	34.6	1.1
80	71	40.2	0.6	45	42.7	1.0

In the preliminary tests, irradiation of older larvae with 920 r had not given any facet reduction, but 1380 and 1840 r had produced a reduction in facet count in those individuals which survived the treatment (see table 1). In the present test, a slight facet reduction resulted from 920 r administered to older larvae. These facts suggest that there is a process or processes acting on facet number in older larvae which is affected by X-rays beyond a certain threshold dose (near 900 r). This radiation effect might be and probably is different from the main effect, a facet reduction induced in larvae irradiated during the sensitive period.

Dosage and mean facet number

The effects of X-rays on mean facet number during this most sensitive period, when the larvae are between 57 and 67 hours of age at the time of irradiation, are shown in table 3 and figure 2. A special experimental test for determining this relationship was run with dosages of 345, 460, 690, 1150, 1380, 1610, and 1840 r. To the data of the above test, all of the results obtained from previous tests which had been made on larvae of the critical age were added. The relationship between mean facet number and X-ray dosage is nearly linearly proportional throughout the range of dosages used. The deviation from the lines are more marked for high doses; this may be attributed to high lethality and small sample sizes. The table and the graph bring out clearly that the effect of the X-rays in reducing facet number is more pronounced per unit dose in males (0.0189 per r) than it is in females (0.0136 per r). In the controls the mean facet number, as is usual in Bar

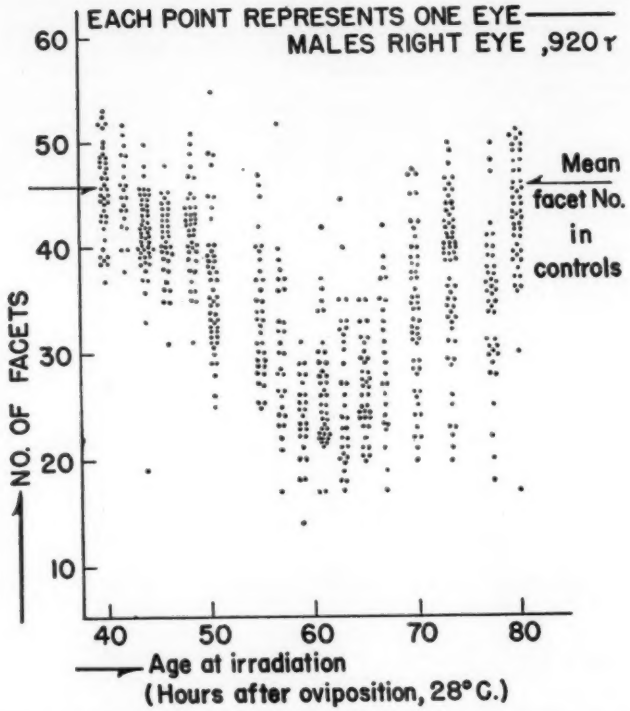

FIGURE 1.—Dot diagram of right eye of males. Larvae treated with 920 r. Temperature 28°C. Number of facets plotted against age in hours at time of irradiation. Data summarized in table 2.

stocks, is lower in females than in males. Due, however, to the more pronounced effect of the X-rays on the eyes of males the curves for the sexes cross each other. It is interesting to note that other agents affecting the facet count (temperature, LUCE 1935; Calliphora extract, CHEVAIS 1943 etc.) show the same sex differences, the males being more affected than the females by equal stimuli.

DISCUSSION

Sensitive periods

Several agents other than X-rays affect facet counts in Bar-eyed flies if applied during the larval stages of development. Among these are temperature (see CHEVAIS 1943, and MARGOLIS and ROBERTSON 1937 for the literature); oxygen (MARGOLIS 1939; LUCE 1939, 1940); formalin (HINSHAW 1941; LUCE 1942); extract from young pupae of the blow fly, *Calliphora*

TABLE 3

Relation between mean facet number and X-ray dosage applied during most sensitive period. Larvae between 57 and 67 hours of age. 28 degrees C.

Dosage in r units	Females				Males			
	No.	Mean facet number	Stan. error	Cal. facet number 0.0136 facets per r	No.	Mean facet number	Stan. error	Cal. facet number 0.0189 facets per r
Controls	452	42.4	0.2	42.4	418	44.6	0.2	44.6
115	35	41.3	0.7	40.8	31	41.6	0.6	42.5
345	37	40.1	0.7	37.7	37	41.6	0.9	38.1
460	181	36.3	0.3	36.1	153	37.1	0.4	35.9
690	20	33.5	0.8	33.0	21	33.9	1.4	31.6
920	363	29.8	0.3	29.8	338	27.2	0.4	27.2
1150	12	24.3	2.1	26.7	16	21.5	2.6	21.7
1380	40	25.2	1.8	23.6	47	19.7	1.8	18.8
1610	33	18.2	2.2	20.5	28	18.6	3.2	14.2
1840	57	18.6	1.2	17.3	48	13.6	1.2	9.6

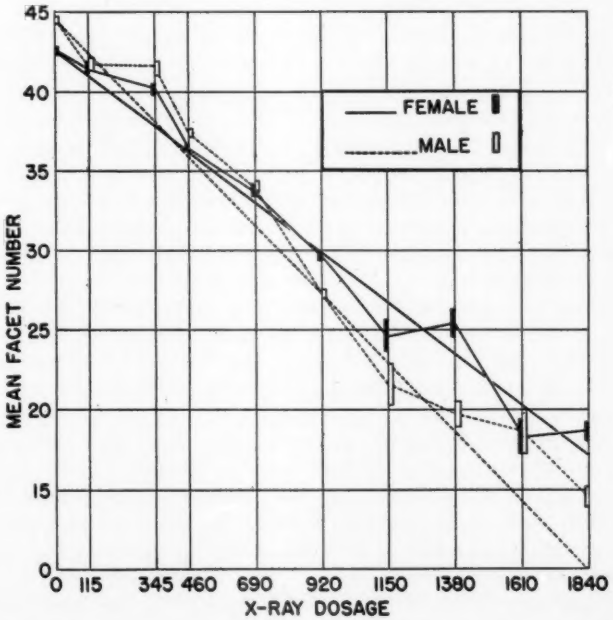

FIGURE 2.—Mean facet number plotted against X-ray dosage. Age of larvae when irradiated between 57 and 67 hours (period of maximum sensitivity). Temperature 28°C. Solid lines females. Broken lines males. Data taken from table 3.

erythrocephala (CHEVAIS 1943). Temperature and formalin, like X-rays, decrease facet number and in general act to intensify the Bar effect of facet reduction. Infrabar is an exception, the facet number in this mutant increasing with rise in temperature (LUCE 1926). The application of oxygen and the administration of Calliphora extract since they both increase facet number, suppress or are antagonistic to the Bar effect. One of the most striking relationships emerging from the study of these environmental agents (except formalin with which critical tests have not been made) is that each produces its major effect when administered during a definite period in larval development. Furthermore, the evidence indicates that this sensitive period is the same for all within the limits of experimental error. These considerations clearly suggest that the environmental agents are producing the major part of the change in facet number by influencing some process or processes concerned with the formation of ommatidia that is in operation during the sensitive period. The results with X-rays, since they show minor changes in facet number occurring when treatments are made both before and after the sensitive period, suggest that there are in addition other processes going on in the larvae at these times which play minor roles in modifying facet number.

Development of wildtype and Bar eye

Any explanation in biological terms of the effects of X-rays on the Bar mutant must rest on a knowledge of the development, growth and differentiation of the compound eye. This problem has been extensively studied by numerous workers (see STEINBERG 1941–1943 and CHEVAIS 1943 for the literature). Early in the developing embryo the ectoderm in the anterior region inpockets to form a single pharyngeal pouch. Near the time the larva hatches from the egg invaginations from the lateral margins of the pharynx begin and growing posteriorly develop into right and left frontal sacs. By the time of 43–45 hours after deposition, the frontal sacs have each developed into two distinct imaginal discs; an anterior, antennal, and posterior, optic disc. By the same time each of the frontal sacs have become attached to the larval brain by the optic stalk. At this stage the entire structure is usually designated as the cephalic complex. The optic disc continues to grow, becomes cup-shaped and is closely applied to the anterior lateral aspect of the larval brain. Throughout all these stages and up to about 80 hours after deposition the cells of the discs have continued to multiply as simple undifferentiated cells without aggregations of any sort. Now for the first time they begin to form aggregations of four cells each, the "goblets" of STEINBERG (1943). These rudiments mark the beginning of visibly differentiated structures which will become the ommatidia of the adult eye. During the remainder of larval life the cells grow slowly and continue to form into such aggregations. During the prepupal stage the median walls of the cephalic complex fuse and disappear and the complex becomes a single large sac with an opening to the outside at the anterior end of the prepupa. The end of the short prepupal stage is marked by the sudden eversion of the complex to form the head of the

pupa. It is evident that the complex by its eversion not only forms the eyes and antennae but also the integument of the sides of the head. The ommatidial rudiments continue to form throughout this period but still consist only of simple aggregations. By the end of the second day of pupal development the aggregations have become more complex, consisting of eight cells with a clear indication of a central rhabdome. In the three-day pupa the ommatidia are nearly completely formed and pigmentation of the eye is beginning.

STEINBERG (1941–1943), CHEVAIS (1943), and LEWIS (1949) have all made careful comparative studies of the development and growth of Bar and wildtype eyes in the larval stages. All conclude that the primordia are measurably smaller and have fewer cells in Bar than in wildtype from the earliest time of their appearance but that the rate of growth, while showing minor variations, closely corresponds in both. The timing of the processes is the same in both. STEINBERG extended his comparative study through the prepupal and pupal stages and found no differences except that there were fewer ommatidial rudiments in Bar.

Correlating the timing of the developmental stages with the temporal location of the sensitive period, it appears that in relation to the formative stages of the eye this period begins shortly after the separation of the antennal from the optic discs and is over before the appearance of the simple aggregations of the cells in the optic disc which mark the first visibly apparent manifestations of the ommatidial rudiments.

The action of X-rays

Irradiation very generally interferes with development. Irradiation of developing embryos often causes anomalies. There is a correlation between the timing of irradiation and the type of anomaly produced. This suggests that irradiation preferentially affects processes which are in some critical stage. In the case under investigation, the developmental stage is characterized as critical not only because of the response, during this stage, to X-rays but by the fact that a number of other environmental agents also have been found to affect the facet number if applied during this stage. In the sections to follow the nature of the processes which make a certain developmental stage critical will be discussed.

X-irradiation notoriously affects mitosis. The mitotic rate of the cells of the optic disc is, however, about the same during the sensitive period as at any other time of larval life. Thus, it is unlikely that the radiation effect on facet count is connected with the radiation effect on mitosis.

The sensitive or critical period occurs between the time of separation of the optic from the antennal discs and the time of the first appearance of the ommatidial rudiments, the "goblets" of STEINBERG. It is during this period that X-rays produce their major effect on facet number as do also the other environmental agents which are known to affect facet number in Bar. As the sensitive period is over just before the first appearance of the initial ommatidial rudiments, the cells of the discs at this time are without much question

going through internal changes in preparation for the visible differentiation which is soon to follow. The X-rays must act by modifying in some way these internal changes.

CHEVAIS' (1943) experiments with Calliphora extract indicate that the cells of discs of the mutant Bar are deficient in a diffusible substance which is necessary for the differentiation of the cells of the optic bud into ommatidia. This substance or some material which can be easily changed into it by the larva is present in the Calliphora extract. If the extract is administered in sufficient quantity the eye of Bar will have as many facets as that of wildtype. The extract to be effective in changing the size of the eye must be administered before the visible differentiation of the ommatidia begins. Administration of the extract to wildtype larvae has no effect on the size of the eye, showing that wildtype larvae normally possess the active principle in sufficient quantity for the process of differentiation to proceed with maximum efficiency.

X-rays might act by effectively reducing still further the small amount of the active principle possessed by the Bar larvae, be it by direct inactivation, or by interference with production or utilization. Whatever the specific nature of the substance may be it is apparent that the X-rays are affecting the chemodifferentiation of the cells which is occurring in preparation for the beginning of visibly apparent differentiation. In other words, some precursor to manifest differentiation is affected which plays a decisive role in the determination of the cells that will become ommatidia.

As to the effect of X-rays on facet number outside the sensitive period, with low dosages (115 r) there was a small increase in facet number due to application during the egg and early larval stage. The increase was very small and did not appear to be temporally localized at any age group but extended over the whole period from 5 to 40 hours. During this time the localization of the frontal sac cells from the pharynx, their invagination to form the sacs and the growth of each posteriorly takes place. The effect cannot be said to be associated with any one of these developmental processes and must be of a general nature occurring through all of them. Turning now to the effect on the older larvae of the 73–85-hour age groups when the larvae are definitely past the sensitive period of maximum response: the dosage response relationship at these later stages was quite different from that during the sensitive period and indicates that the X-rays are acting on a different process than the one acted on during the sensitive period. Since visible differentiation of the optic rudiments in the eye discs is now taking place it seems plausible to assume that the X-rays are acting by slightly inhibiting the actual process of differentiation.

The antennal bud is a part of the same structure as the optic bud up to approximately the time when the radiosensitive period sets in. It is of interest that the antennae are not affected by the environmental and genetic factors which change the facet number.

Action of the Bar mutants

The comparative study of the eye of the Bar mutant and in the wildtype shows that there are two widely separated developmental stages in which major differences are found that can be said to be due to the action of the mutant Bar. The first demonstrable difference is that the eye primordia, at the earliest stage at which they have been definitely identified, are smaller and contain fewer cells in Bar than in wild type. The initial effect of the Bar mutant must occur in the processes that localize and determine the cells which will form the frontal sac. That such determination is actually taking place at this time is indicated by the experiments of HOWLAND and CHILD (1933) and PATTERSON (1929). By puncturing the egg 4–8 hours after deposition and destroying some of the cells in the left lateral and dorsal mid-line region, HOWLAND and CHILD found that imagoes with missing and abnormal eyes resulted. PATTERSON found that when X-rays were applied early (0–12 or 12–24 hours) the size of the resulting somatic mutant areas in the eyes was greatest in extent. In one case both eyes were involved and in several others eyes of half mutant tissue were obtained. The early effect has not been studied experimentally in Bar.

The initial size difference between Bar and wildtype optic rudiments is maintained throughout larval life by their similar rates of growth. The size difference between the eye discs of Bar and wildtype has probably no decisive effect on the sizes of the definitive eye though minor differences in facet number might be accounted for in this way. This interpretation receives support from the results of STEINBERG (1941b) who found that the $m(B)$ factor did not measurably affect the size of the Bar disc when it was present in the genotype. The magnitude of the increase in facet number in the adult eye which could be ascribed to the $m(B)$ factor was certainly great enough to be detected in this way if disc size always foreshadowed accurately the size of the eye. The secondary role of disc size in determining the size of the adult eye is further attested by CHEVAIS' tests (1943) with Calliphora extract. He showed that Bar discs though definitely smaller than wildtype were capable when treated with purified extract of producing an eye as large as wildtype.

The second stage in development when the Bar mutant differs from wildtype is during the sensitive period. Bar mutants when compared to wildtype under normal conditions always produce fewer ommatidia and more integument or head chitin. That this difference is brought about by the action of the cells of the Bar mutants during the sensitive period is indicated by the effects of environmental agents upon the expression of the Bar alleles when applied during this period. If the agent employed reduces the number of ommatidia it increases the amount of head chitin and vice versa. The cells of the Bar mutants are deficient in a substance which is a necessary precursor for the initiation of differentiation into ommatidia. This deficiency may be brought about either by suppression or inactivation of the substance in the cells of the Bar discs or more probably simply because these cells produce it

in insufficient amounts. The various mutants of the Bar series and their heterozygous combinations manifest the deficiency in varying degrees and thus account for the facet numbers of the phenotypes which are characteristic of each of the Bar mutants and each of their heterozygous combinations.

The two effects of the Bar mutants though widely separated in developmental time are fundamentally similar. In the initial effect the process of localization of the original optic rudiment is affected, fewer cells become determined among the cells of the pharynx as cells which will develop into the frontal sacs. In the second case fewer cells of the optic bud become determined to differentiate into ommatidia and are left to form head chitin instead.

SUMMARY

Larvae of Bar-eyed Drosophila, kept at a temperature of 28°C, which were irradiated with X-rays of dosages varying from 115 to 1840 r, developed into adult flies whose compound eyes had a smaller number of facets than untreated controls.

With a dosage of 920 r the effect of the X-rays was largely confined to a radiosensitive period extending from about 55 to 70 hours of egg-larval life with a maximum reduction occurring between 57 and 67 hours.

The radiosensitive period closely corresponds to similar sensitive periods for other environmental agents affecting facet number in Bar-eyed Drosophila.

The reduction in facet number following application of X-rays during the sensitive stage was nearly proportional to the dosage applied.

In males the reduction in facets per unit dose was always greater than in females.

The biological effect of the X-rays and the nature of the mode of action of the Bar mutants are discussed.

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VIABILITY AND SEX DETERMINATION IN THE HONEY BEE (APIS MELLIFERA L.)

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AFTER WHITING (1940a, 1943) established conclusively that sex in the parasitic hymenopteron Habrobracon is controlled by multiple sex alleles, it became of interest to determine whether the same mechanism exists in other insects, in which males develop from unfertilized eggs and are haploid. The work described in this paper was started in 1944 at the SOUTHERN STATES BEE CULTURE LABORATORY, of the BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE, to determine whether sex is controlled by the same mechanism in the honey bee. In this insect the problem was also of practical interest because it involved egg hatchability, which is of vital importance in the building up of colony populations for honey production.

According to WHITING, sex in *Bracon hebetor* Say [2] is controlled by a series of sex alleles (*xa, xb, xc,* etc.). Females are heterozygous (*xa/xb, xa/xc,* etc.), and when unmated produce haploid sons of two types in equal numbers (*xa* and *xb, xa* and *xc,* etc.). When females are mated, about two-thirds of their eggs are fertilized. If the parents have different sex alleles (*xa/xb* × *xc,* for example), all fertilized eggs develop into females (*xa/xc* and *xb/xc*). This is called a three-allele fraternity. If one of the sex alleles of the female parent is identical with that of the male parent (*xa/xb* × *xa* for example), then one-half the fertilized eggs are heterozygous (*xa/xb*) and develop into females, and one-half are homozygous (*xa/xa*) and highly inviable but sometimes develop into adult biparental males. This is called a two-allele fraternity. Haploid males are highly viable. Inbreeding tends to bring together identical sex alleles, resulting in low viability, whereas in outcrosses such combinations are less likely and good viability usually occurs. WHITING has already demonstrated nine alleles, and there are probably many more present in the wild population. Their allelic nature has been demonstrated by linkage tests.

Although *Bracon hebetor* is the only species in which the existence of multiple sex alleles has been proved, genetic work suggests that the sex-determining mechanism is the same in other members of the genus and in other Hymenoptera in which outcrossing is common. In *B. brevicornis* Wesmael, SPEICHER and SPEICHER (1940) found biparental males. INABA (1939) has

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[2] Usually treated in WHITING'S papers under the name *Habrobracon juglandis* (Ashm.). On account of its familiarity to geneticists the term "*Habrobracon*" is retained in this paper.

reported biparental males and evidence for multiple sex allelism in *Habrobracon pectinophorae* Watanabe, but this species seems to be identical with *Bracon hebetor* (WHITING 1949). In the chalcidoid wasp *Pteromalus puparum* (Linnaeus) genetic evidence obtained by DOZORCEVA (reviewed by WHITING 1940b) suggests two- and three-allele fraternities and multiple alleles. In these forms, in which outcrossing is the rule, the waste of reproductive effort through low viability would not be a serious matter. In close-crossed species, however, where frequent two-allele fraternities would cause a great waste of eggs, the multiple-allele scheme does not seem to apply in the species that have been studied. In Melittobia, in which mother-son and sibling matings are the rule with rare outcrossing, SCHMIEDER and WHITING (1947) found neither diploid males nor low viability in close crosses—an indication that the method of sex determination may be different. In another naturally close-crossed species, *Telenomus fariai* Lima, DREYFUS and BREUER (1944) report no biparental males and present cytological data to show that females are heterozygous for sex, having X and Y chromosomes which are distinguishable. The males developing from unfertilized eggs have an X chromosome in all the somatic cells and in the early spermatogonia. In spermatogenesis the X is reduced to a Y by loss of a fragment, so that all sperms are Y-bearing. It is assumed that through differential maturation in oogenesis the Y passes into the polar body and that the egg pronucleus always receives an X. MANNING (1949) describes a similar mechanism in the honey bee, except that there is no Y chromosome. All eggs become X-bearing through differential maturation, and in spermatogenesis the X is extruded so that all sperms contain only a set of autosomes. Two sets of autosomes plus an X determine femaleness, and one set of autosomes plus an X determines maleness.

Since the honey bee is naturally outcrossed, we should expect its sex mechanism to conform more closely to that of Habrobracon than to that of close-crossed species. This paper describes viability tests and gives relevant data that suggest conformity to the Habrobracon scheme.

MATERIALS AND METHODS

If in Habrobracon we start with a three-allele mating, such as $xa/xb \times xc$, and make individual sibling matings for two generations, viability will follow a definite pattern. In the first generation one-half of the fraternities will be the two-allele type, exhibiting low viability of zygotes, and one-half will be of the three-allele type, exhibiting high viability of zygotes. In the second generation, if we breed from a low-viability (two-allele) fraternity, only low-viability fraternities will result, and if we breed from a high-viability (three-allele) fraternity, the results will be the same as in the first generation.

The same plan was followed with the honey bee. A naturally mated queen producing progeny of high viability was chosen. Daughter queens were mated individually to their brothers, and the worker progeny (from fertilized eggs in worker cells) tested for viability to the fifth day. Granddaughter queens

were then reared from one of the daughters producing highly viable worker progeny and from one producing poorly viable worker progeny, and mated individually to their brothers. The resulting worker progeny were tested for viability to the fifth day, and some to the nineteenth day just before emergence. The nineteenth-day counts were not made at random, but in all tests conducted after a certain date.

Mating was controlled as completely as is possible in the honey bee. All matings were by artificial insemination by the method described by MACKENSEN and ROBERTS (1948). The insemination syringe was sterilized between matings. All drones were reared in colonies stocked with bees of a distinguishable color, so that drones from laying worker bees could be recognized and discarded.

A viability test was made as follows: When the queen started laying, the nucleus (small colony) was strengthened with bees and brood if this seemed necessary. The queen was caged in an excluder cage (one which permits the passage of bees but not of the queen) on a specially prepared or selected comb. Such a comb was usually taken from another nucleus—a comb in which the queen had not yet started laying but in which the worker bees had already polished the cells in a well-defined area surrounded by pollen. When such combs were not available, a clean comb was prepared by adding pollen in a ring around the central area. The queen was restricted to one side of the comb for 24 hours and then to the other side for a similar period. The excluder cage was then removed and the queen confined in a small screen cage hung in the nucleus to prevent excessive production of queen cells. At the end of each 24-hour laying period the eggs were counted in a rectangular portion of the laid area, and the larvae were also counted on the fifth day. At the last count the oldest larvae were therefore five days old and the youngest four days old. Eggs normally hatch in 76 hours, but it is so difficult to see them that they cannot be counted in great numbers.

The viability tests on the second generation were made without the use of an excluder cage. A nucleus hive containing three standard Langstroth frames was used. As soon as the queen was laying well, the center comb was simply replaced with a selected or prepared test comb. Providing side combs solidly filled with honey and pollen forced the queen to lay in the test comb. After the queen had layed for 24 hours, the eggs were counted. Counts were again made on the fifth and nineteenth days.

These counts do not record the time of death, but the number of individuals remaining apparently alive at the various stages. Although nurse bees remove dead remains quickly, there is certain to be some delay. In these tests shriveled eggs and dead larvae or pupae were rarely seen, and a check of sealed brood showed that a count of the normally capped cells on the nineteenth day was accurate.

Viability tests were also made on drone eggs (unfertilized). Since these tests were made during the summer, when queens are unlikely to lay drone eggs and bees are unlikely to care for them properly, special methods were

necessary. Special combs were prepared by inserting rectangular pieces of drone comb about 3 by 4 inches in the center of a worker comb. Pollen was packed into the cells at both ends of each comb, so that only a small area of empty worker cells surrounded the drone cells and the queen was thus forced to lay in them. In other respects the method was similar to that described for worker brood. Often several days elapsed before eggs were laid in the drone comb.

Mated queens ordinarily lay only fertilized eggs in worker cells and unfertilized eggs in the larger drone cells. However, artificially mated queens sometimes receive so few sperms that some of the eggs laid in worker cells are not fertilized. Such queens are called partial or complete drone layers, as the case may be, and can be recognized by the appearance of the cappings of sealed brood, the cappings over drone larvae in worker cells being more rounded than those over worker larvae. Eleven such queens were excluded from the worker brood viability data.

A queen forced to lay on drone comb will often lay fertilized eggs. As in worker comb, worker larvae are capped flat and drone larvae rounded. Rarely was a flat capping found in the drone brood tested in these experiments.

RESULTS

Viability tests on worker brood

Of the eight queens tested in the first generation, four produced brood of low viability to the fifth day—45, 45, 48, and 49 percent; the other four produced brood of high viability—90, 95, 96, and 97 percent. At least 742 eggs of each queen were counted.

The second-generation queens are divided into two groups: (1) daughters of a first-generation queen producing brood of low viability—45 percent on the fifth day; and (2) daughters of a first-generation queen producing brood of high viability—90 percent on the fifth day. The results are given in table 1. In the first group all 34 progenies tested were poorly viable, ranging from 43 to 66 percent, with an average of 51 percent. Twenty-seven tests carried to the nineteenth day revealed brood ranging from 32 to 49 percent in viability, with an average of 43 percent. Progenies of the second group fell definitely into a high and a low class, as in the first generation, with 10 in the low class and 16 in the high class. On the fifth day the low class ranged from 41 to 59 percent (average 52), and the high class from 88 to 97 percent (average 94). On the nineteenth day the corresponding figures for tests carried to this stage were 34 to 46 percent (average 43), and 85 to 97 percent (average 91).

The nineteenth-day counts showed that total viability remained in the high class when the fifth-day count was in the high class, and that the nineteenth-day viability was definitely low in tests in which the fifth day count was in the upper part of the low-viability class. By this time the two highest fifth-day counts (66 percent each) were reduced to 34 and 48 percent.

To determine at what age death usually occurs, more frequent counts were made on brood of two queens producing low-viability brood. The results are

TABLE 1

Viability of worker brood produced by second-generation queens.

Brood of daughters of a first-generation queen producing poorly viable brood			Brood of daughters of a first-generation queen producing highly viable brood		
Number of eggs	Percentage surviving		Number of eggs	Percentage surviving	
	5th day	19th day		5th day	19th day
125	66	34	528	53	....
714	55	....	484	54	46
577	50	49	621	52	34
980	48	46	674	51	45
854	54	41	819	54	....
696	49	39	726	57	45
501	50	43	849	53	....
936	49	46	810	41	39
279	55	32	903	49	46
593	66	48	943	59	....
371	49	47	....	....	....
911	50	....	....	....	....
566	54	35	975	96	93
328	52	....	370	95	88
484	50	....	765	93	90
804	46	45	921	97	97
774	49	47	696	96	....
600	48	45	857	97	96
825	51	48	649	93	91
446	50	....	884	94	89
562	43	40	518	88	86
688	48	45	863	97	94
415	47	43	514	93	92
418	50	47	763	93	....
259	45	....	859	94	....
216	49	40	319	91	87
720	53	44	428	97	96
725	49	47	344	95	85
602	49	44	....	....	....
851	48	....	....	....	....
487	61	42	....	....	....
649	48	47	....	....	....
900	50	49	....	....	....
660	57	36	....	....	....

given in table 2. The greatest loss occurred between the third- and fourth-day counts or just at the time of hatching, as in Habrobracon. The viability did not drop below 50 percent until after the ninth day. Apparently some of the homozygotes live to this stage.

Viability of drone brood

Tests were made on drone brood to determine the viability of unfertilized eggs. Two tests on the drone brood of a queen whose worker brood was 45 percent viable on the fifth day gave the following results: 232 eggs tested 94 percent viable, and 261 eggs tested 83 percent viable. Three tests on drone brood of another queen, whose worker brood tested 48 percent viable on the

TABLE 2

Percentage survival of low-viability broods from two queens.

Queen No.	Number of eggs	Days after beginning of test								
		2	3	4	5	7	9	11	13	19
S-8	484	98	95	60	54	53	52	47	46	46
S-17	726	97	94	59	57	56	52	47	46	45

fifth day, gave the following results: 188 eggs tested 65 percent viable; 164 eggs, 90 percent viable; and 229 eggs, 74 percent viable. In low-viability fraternities, therefore, unfertilized eggs are more viable to the fifth day than fertilized eggs.

Results are also available from tests on the brood of six mated queens that produced nearly 100 percent of drone brood. The intention being to test fertilized eggs before it was known that the queens were drone layers, the tests were conducted in worker combs. The number of eggs tested and the per cent viable to the fifth day in these tests were as follows: 219, 87 percent; 244, 83 percent; 341, 89 percent; 167, 91 percent; 369, 88 percent; and 230, 90 percent. Later counts were not attempted because many drones die from lack of room in worker cells. Data from all these drone-brood tests show that viability is associated with fertilization, as in Habrobracon.

Tests for biparental males

In Habrobracon biparental males can occur when identical sex alleles come together in a mating. They are phenotypically recognizable when a recessive female is mated to a wild-type male. In such a mating uniparental haploid male progeny are recessive and the biparental diploid males are wild-type. In the honey bee a further requisite for the absolute identification of biparental males is that they be reared in a colony stocked with bees of a contrasting body color, so that the haploid sons of wild-type laying workers, which occasionally occur in any normal hive, can be distinguished from the diploid biparental sons of the queen.

The first attempt to recognize biparental males was made in a line carrying the recessive mutant white, causing a complete lack of pigment in the eyes. This line was maintained by sibling matings in such a way that biparental males could be recognized every other generation. No such males were found, although for several generations thousands of biparental individuals emerging from worker comb were examined for each generation. In this material conditions were not the most favorable, because it was not certain that the line had been reduced to two alleles.

Another attempt was made in a stock carrying the recessive mutant cordovan, which changes the black body pigment to various shades of brown, cordovan appearing in the thorax. This stock was supplied by the DIVISION OF BEE CULTURE, Beltsville, Maryland. Heterozygous queens were mated individually to their brothers, and a two-allele mating, one producing brood

of low viability, was then chosen to continue the line. Homozygous cordovan daughters were then mated to wild-type brothers. Viability of the worker progeny of these matings was low, the line having apparently been reduced to two alleles as expected. Biparental males were expected to be of the wild type in contrast to the cordovan of the usual haploid males. The progeny of two queens were examined. The queens were caged on a fresh comb every few days. Drone comb was used, so that all abnormal individuals, biparental males, haplo-diploid males, or sex mosaics had the advantage of developing in large cells. Of the 12,533 individuals examined, 8,603 were wild-type workers, 3,929 were cordovan drones, and 1 was a sex mosaic with the female parts wild-type and male parts cordovan.

DISCUSSION

The parallelism between Habrobracon and the honey bee brought out in these experiments strongly suggests that the mode of sex determination is the same in the two insects. At least there seems to be in the honey bee a series of haploviable homozygous lethal alleles similar in lethal action to the sex alleles of Habrobracon. That such a lethal mechanism is at work, and that it is probably universal in the honey bee, lending support to its association with sex, is amply substantiated by experience in bee breeding since these experiments were made. Mother-son (two-allele) matings in material from various sources representing two races have invariably resulted in low viability. Two-allele lines established by such matings have continued to show low viability and to breed as two-allele lines. Crosses by individual mating between two-allele lines fall into one of three classes as expected: (1) all progenies of low viability, showing that the alleles are the same; (2) all progenies highly viable, showing the alleles to be different; and (3) one-half the progenies highly viable and one-half poorly viable, showing that the two lines have one allele in common. This procedure is now being used to establish two-allele tester lines with definitely identified alleles for use in determining the alleles of any unknown breeding stock.

Although biparental males, which are necessary to prove a relation to sex, were not found, apparently many of the homozygotes live to various stages of larval life. In low-viability broods the survival counts on the fifth day average higher than 50 percent, and in table 2 the ninth day counts are higher than 50 percent. Perhaps by selection a strain can be developed in which some live to maturity.

If sex is determined as MANNING suggests, then the lethal alleles must be autosomal and have no connection with sex. It is difficult however to imagine the survival of such a wasteful series of lethals unless it is associated with something as vital as sex.

Since inbreeding often brings about low viability and a waste of eggs and young larvae, we would expect the honey bee to have developed behavior patterns that promote outbreeding. This is the case. Mating does not take place in the hive where severe inbreeding would result, but on the wing where

drones from other colonies are available. In the wild state bees live in hollow trees and cliffs and are widely scattered. A queen mating near her own hive would probably meet a drone from her own colony—one of her brothers. Mating at a distance would increase the chance of meeting a drone from another colony. Some observations indicate that virgin queens do mate at some distance from their own colony.

In a favorable open location fifty virgin queens were followed with the eye until they either met drones or passed beyond the range of vision. After leaving the hive the virgins flew in ever-increasing circles until a radius of about 50 feet was reached, and then headed straight away from the queen yard. Two of the virgins were caught by drones while still circling and they dropped toward the ground, but neither drone nor queen could be found on the ground. The others were followed closely by one or more drones as they circled, but as they headed away from the queen yard they invariably were alone until they disappeared from view. From these observations it appears that they deliberately avoid drones near their own hive.

PRACTICAL CONSIDERATIONS

One of the main factors in the honey production of the bee colony is its population, which in turn is largely dependent upon the laying capacity of the queen and the efficiency within the brood nest. The number of eggs a queen can lay in a day is limited, and if half her eggs and young larvae die, as in a two-allele mating, the queen does not have the capacity to lay the additional eggs required to maintain the colony at full strength. In addition, the efficiency within the brood nest is reduced when half the eggs and young larvae occupy cells for three days or longer only to die and be removed. The brood becomes spotted and the queen cannot follow her regular laying pattern, but must hunt for scattered empty cells. This inefficiency may be a very critical factor early in the spring, when the brood nest is limited to a small space that the bees can keep warm.

The fact that about one-half of all queens mate a second time (ROBERTS 1944) reduces the amount of very low viability, because under the usual conditions existing in queen yards a queen is not likely to meet a drone with a similar lethal allele at both matings. Theoretically, the brood of twice-mated queens ranges in viability from 50 to 100 percent depending on the types and proportion of types of sperm received. Although minimized by double mating, the effect of lethal alleles is still serious, especially because the reduction in efficiency is often too slight to be readily recognized by the beekeeper.

It is therefore greatly to the beekeeper's advantage to have his queens properly mated as to lethal alleles. He must avoid inbreeding and select for good brood quality to keep as many alleles in the strain as possible. The most convenient way for the queen producer to do this is to be sure that, for each generation, the breeder queens (already mated) he uses as a source of virgins are descended from different mothers than those he uses as a source of drones. Each season he should use several breeder queens for the production of vir-

gins and several for the production of drones. When too much brood of poor quality appears, he can outcross to an unrelated strain. A system of criss-crossing strains might be advantageous. Finally, with the aid of artificial insemination, he can establish and maintain inbred lines containing known sex alleles and then cross them in the best combination. The last method is beyond the facilities of the individual breeder, but can be carried out by cooperative and agricultural research agencies which can distribute foundation stock for the production of hybrids by the commercial queen producer.

SUMMARY

Experiments were conducted at the SOUTHERN STATES BEE CULTURE LABORATORY, of the BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE, to ascertain whether the sex-determination mechanism in the honey bee (*Apis mellifera* L.) is similar to that of the parasitic hymenopteron Habrobracon, in which there exists a series of sex-determining alleles, *xa, xb, xc,* etc. Heterozygotes are females, azygotes males, and homozygotes biparental males which are highly inviable.

Evidence was presented which supports the existence of a similar mechanism in the honey bee. When individual sibling matings were made for two generations, the viability of the worker progenies followed the same pattern expected if similar matings were made in Habrobracon. Although the data obtained show the existence of a series of haploviable homozygous lethal alleles similar in their lethal action to the sex alleles of Habrobracon, a relation to sex was not established because biparental males were not found in crosses of homozygous recessive females to wild-type males.

In sex determination the outcrossed honey bee is apparently more like Habrobracon and other outcrossed Hymenoptera than like the close-crossed species in which neither biparental males nor low viability occur in close crosses.

Observations are recorded which indicate that queens have a natural tendency to mate away from their own hives, thus avoiding inbreeding. Since inbreeding brings together similar lethal alleles and lowers brood viability, colony population, and honey production, it should be carefully avoided in commercial beekeeping practice.

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ON THE MUTABILITY OF THE WAXY LOCUS IN MAIZE*

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THE genetic analysis of mutation represents one of the classic lines of investigation of the nature of the gene. A serious difficulty with this approach has been the rare occurrence of spontaneous mutation at most gene loci. As a consequence, geneticists have sought methods to speed up mutation rates artificially, by various means, notably irradiation and chemical treatment, and recently selection of genetic modifiers (STADLER 1946, 1948, 1949).

The way was paved for a different attack upon this problem by the work of EMERSON with a strain of maize that was variegated for colored and colorless pericarp. He showed (EMERSON 1914, 1917, 1929) that the variegation was inherited as if due to an unstable form of the gene for colored pericarp, and that the self-colored kernels found occasionally on variegated ears, were attributable to mutation of the unstable form to a stable dominant allele.

Unstable genes in Drosophila and Delphinium were studied by DEMEREC. His results, together with those of other workers have been summarized in a review article (DEMEREC 1935).

In a series of investigations on the dotted gene in maize, RHOADES (1936, 1938, 1941a, 1941b, 1945) established the occurrence of a unique sort of instability. In this material, a dominant allele at the dotted locus on chromosome 9 specifically induces the mutation of a recessive allele at the a_1 locus on chromosome 3.

The work to be presented in this paper deals with another unstable gene in maize, called mutable-waxy. It was found by HUTCHISON in 1921, in an ear resulting from the pollination of a plant supposed to be non-waxy ($Wx\,Wx$). This ear contained some kernels mosaic for the waxy phenotype, *i.e.*, some endosperm cells stained red with iodine, a test for the recessive waxy phenotype, while others gave the blue-black stain produced in the presence of the Wx allele.

Preliminary studies were made of this mosaic material by EMERSON, by DEMEREC, and later by RHOADES (all unpublished). From their data, it appeared that the mosaicism might be due to an unstable recessive allele at the waxy locus, which arose by mutation from Wx and which mutated to the dominant form, presumably, in both somatic and germinal tissues, at an extremely high rate.

* The cost of the accompanying plate has been paid by the GALTON AND MENDEL MEMORIAL FUND.

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This material was of interest for several reasons. In the first place, the waxy locus has been noted for its extreme stability. In the forty years that this locus has been used as a marker by geneticists, less than ten distinct, independent mutations (from dominant to recessive) have been reported (ANDRES and BASCIOLLI 1941; BEAR 1944; BREGGER 1928; COLLINS 1920; MANGELSDORF 1924). STADLER (1930) studied mutation rates of the dominant alleles at eight different loci in maize, and found mutations at all loci except the waxy, where none was found in almost two million gametes examined.

Presumably related to this stability is the paucity of alleles known at the waxy locus. Only two (*Wx* and *wx*) were known until 1941 when ANDRES and BASCIOLLI described waxy-argentine (wx^a), an allele very similar to the recessive, *wx*. There are several phenotypic differences attributed to the waxy alleles. Of these, the difference in relative amounts of the two types of starch molecules (amylose and amylopectin) present in endosperm starch has lent itself best to qualitative and quantitative differentiation. As a result of recent advances in starch chemistry (summarized by MEYER 1942; HASSID 1943) and due especially to the potentiometric titration method of BATES, FRENCH and RUNDLE (1943), the effect of various combinations of waxy alleles on the percent amylose in endosperm starch has been measured with considerable precision (SPRAGUE, BRIMHALL and HIXON 1943; BRIMHALL, SPRAGUE and SASS 1945).

It was expected that stable mutants might be recovered from this unstable stock, and if so, the titration method could be used to characterize them with respect to amylose-amylopectin ratio. With this analytical tool, new alleles, not visually distinguishable could be detected, and in view of the apparently high mutation rate, it was hoped that the full mutational potentialities of the waxy locus might be expressed.

Related to the usefulness of this locus for studying allelism, is the fact that both mutation and gene action are accessible to investigation. Most studies of mutation have not been concerned with the metabolic role of the gene and similarly most gene action analyses have not simultaneously considered the mutational behavior of the locus in question. It seemed that a joint attack upon both the mutational and metabolic behavior of a single locus might be fruitful, and that the waxy locus provided material for such an approach.

DESCRIPTION OF THE MATERIAL

1. *Phenotypes of the stable alleles*

The waxy locus became known when COLLINS (1909) found the recessive allele in some corn growing in China. He named the locus "waxy" because of the waxy, opaque appearance of kernels homozygous for the recessive allele, *wx*. That waxy and non-waxy kernels could be distinguished by the iodine staining reaction of their endosperm starch was reported by WEATHERWAX (1922). Subsequently, DEMEREC (1924) and BRINK and MACGILLIVRAY (1924) found that red-staining and blue-staining pollen grains were pro-

TABLE 1

Phenotypic differences attributed to the waxy alleles.

	Wx	*wx*[a]	*wx*	
Genetic classification	Dominant	Recessive to *Wx*	Recessive	
Appearance of dried kernel	Translucent (in corneous stocks)	Opaque	Opaque	
Iodine-staining reaction				
Endosperm	Intense Deep blue-black	Pale Reddish-lavender	Pale Reddish-brown	
Pollen	Deep blue-black	Greyish	Pink to Reddish-brown	
Starch composition				
Amylose	About 28%	2.4%	0%	SPRAGUE, BRIMHALL & HIXON (1943) BRIMHALL, SPRAGUE & SASS (1945)
Amylopectin (by difference)	" 72%	97.6%	100%	
Rate of penetration of pollen into silks	*Wx* penetrates 3.8 minutes faster than *wx*			SPRAGUE (1933)
Pollen viability	*Wx* pollen has lower viability than *wx*			KEMPTON (1927; 1936)
*Kernel weight	Dried *Wx Wx Wx* kernels weigh 2–5% more than *wx wx wx*			COLLINS & KEMPTON (1913) KIESSELBACH (1944)
*Fat content endosperm	*Wx Wx Wx* endosperm contains 40% less fat than *wx wx wx* " " " 30% " " " "			ABEGG (1929) SPRAGUE & BRIMHALL, Unpubl.
Niacin content	*Wx Wx Wx* kernels contain less niacin than *wx wx wx*			RICHEY & DAWSON (1948)

* As summarized in HIXON & BRIMHALL 1944 (see for further bibliography).

duced in equal numbers by *Wx wx* plants. Both the haploid pollen grain and embryo sac contain starch of a composition determined by the particular waxy allele present. Starch of the maize embryo is always blue-staining regardless of the waxy genotype, as is starch found in other parts of the sporophyte, such as leaf and root-cap.

The naturally occurring granular starches that stain blue with iodine are mixtures of two types of molecules, amylose and amylopectin, according to the two-component theory of starch structure (MEYER 1942; HASSID 1943; SCHOCH 1945). Waxy corn starch, which stains red with iodine, is reported to consist 100 percent of amylopectin, while endosperm starch from non-waxy kernels (*Wx Wx Wx*) is reported to contain about 28 percent amylose, the rest being amylopectin. According to BRIMHALL, SPRAGUE and SASS (1945), endosperm starch of kernels homozygous for wx^a contains 2.4 percent amylose, and by difference, 97.6 percent amylopectin.

Other differences besides starch composition have been demonstrated between waxy and non-waxy phenotypes. These, as well as the principal phenotypic differences, are summarized in table 1. In this investigation, the criterion for allelic differentiation has been limited to starch composition.

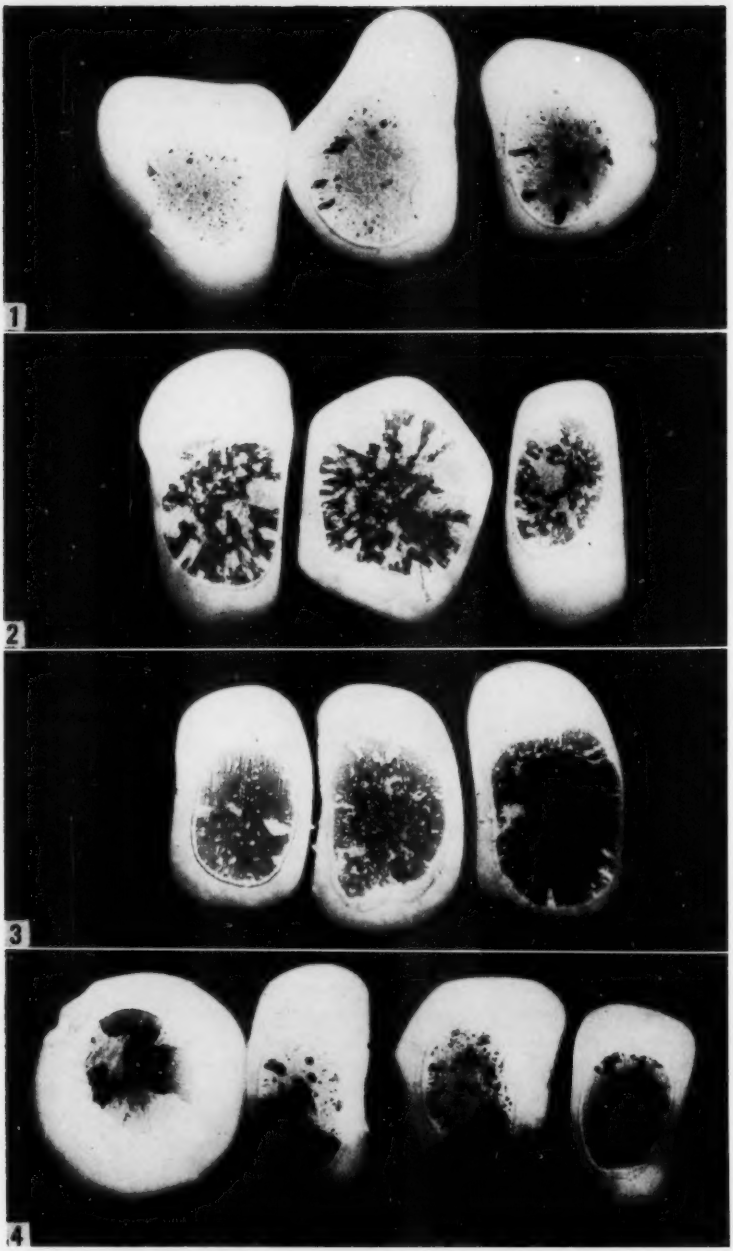
2. *The mosaic phenotype*

When the endosperm of a mosaic kernel is observed closely, some areas appear opaque, and others relatively translucent. If dilute iodine (I_2–KI) is applied, the opaque areas turn red, and the translucent areas blue. It then becomes evident that the iodine-staining property is cell-specific. A blue-staining area consists of a cluster of blue-staining cells; the area ends abruptly at the outermost cell walls. The same is true of the red-staining areas. Individual cells contain all red-staining starch grains, or all blue-staining ones, but never a mixture of the two.

When a section of mosaic endosperm is observed under magnification (36×), small clusters of cells which are intermediate in staining color (lavender) are sometimes found. They, too, are clearly demarcated at the cell wall from adjoining cells which may be either red-staining or blue-staining.

Mosaic patterns of all sorts have been observed. A few characteristic types are shown in figures 1–4. In each case, the outer cell layers have been filed away, exposing endosperm tissue, which has been stained with dilute iodine solution. The intense blue-staining areas appear dark; the pale red-staining areas appear light. These pattern-types were selected from the veritable continuum which have been observed, ranging from kernels with less blue-staining spots than those of figure 1 to kernels with less red-staining areas than those of figure 3.

In addition, sectored kernels have been found in which one area of the kernel has an entirely different pattern from another. The dividing line is often sharp, as in figure 4. Although the most striking kernels are those which are sectored half-and-half, small sectors of differing pattern are also common. These patterns will be considered further in the section: "Mutability as a Phenotype."



FIGURES 1–4.—Mature kernels, sectioned and stained with I_2–KI to show mosaic distribution of starches. Red-staining (waxy) starch appears light; blue-staining (non-waxy) starch appears dark.

Tassels from plants carrying the factor for mosaic waxy endosperm were found to contain both red- and blue-staining pollen grains. Kernels of the type shown in figure 3 gave rise in most cases to plants with tassels containing almost entirely blue-staining pollen. The pollen of plants from kernels of other types exhibited great diversity in ratios of blue-staining to red-staining pollen from individual anthers. The distribution of ratios did not indicate the presence of clear-cut sectors. The percent of blue-staining pollen was high and extremely variable. Both blue-staining and red-staining pollen grains were of normal size and appearance.

No mosaic pollen was found (*i.e.*, single pollen grains containing both red-staining and blue-staining starch grains). This is consistent with the observation that no mixtures of red- and blue-staining starch grains were found within single endosperm cells.

THE MUTATION HYPOTHESIS

On the basis of earlier observations it seemed plausible that mosaic kernels were due to somatic mutations, and the non-waxy kernels found on ears predominantly mosaic, resulted from germinal (pre-fertilization) mutations. On this hypothesis, the tremendously high mutation rate would be attributed to an unstable form of the waxy gene, wx^m, which mutates in both sporophyte and endosperm, and which arose by mutation from *Wx*, the dominant allele. Experiments were carried out to test this hypothesis.

1. *Linkage relations*

There was the possibility that a gene at some other locus was inducing mutability of a normally stable waxy allele. RHOADES (1936, 1938, 1941a, 1945) had demonstrated that the presence of a dominant allele, *Dt*, at the dotted locus in chromosome 9, induced a high mutation rate specifically at the a_1 locus in chromosome 3. The possibility that a similar situation obtained in this material, was tested by outcrossing and by linkage studies.

Plants carrying the presumed wx^m allele were outcrossed to sixteen unrelated maize stocks. In every case, the wx^m factor was recovered in the F_2 generation in the ratios expected from the segregation of alleles at a single locus.

Further evidence comes from linkage data, obtained by test-crossing plants heterozygous for *wx* and wx^m and for genes at neighboring loci on chromosome 9. The following cross was made:

$$\frac{c\ sh\ wx\ v_1}{c\ sh\ wx\ v_1} \times \frac{C\ Sh\ wx^m\ V_1}{C\ Sh\ Wx\ V_1}$$

Mosaic F_1 kernels were selected and test-crossed again:

$$\frac{C\ Sh\ wx^m\ V_1}{c\ sh\ wx\ v_1} \times \frac{c\ sh\ wx\ v_1}{c\ sh\ wx\ v_1}$$

TABLE 2

Linkage data.

F_1 *genotype* $\frac{C\ Sh\ wx^m\ V_1}{c\ sh\ wx\ v_1}$. *(Only colorless kernels classified).*

	No. kernels	% recomb.	% cross-overs	Maize linkage summ. % cross-overs	
				Av.	Range
Non-crossovers	1484				
Crossovers					
Region 1	104	3.8	5.0	3.3	2.3–5.8
Region 2	717	26.4	29.1	21	20–30
Region 3	328	12.1	14.4	12	8–14
Regions 1 + 2	15	0.55			
Regions 1 + 3	5	0.18			
Regions 2 + 3	48	1.77			
Regions 1 + 2 + 3	11	0.41			

Since only the colorless kernels (ccc) could be classified accurately, eight classes are included in the data of table 2 instead of the sixteen expected in a four-point test. Non-waxy kernels, found in both cross-over and non-cross-over classes, were classified with mosaic kernels as wx^m, on the basis of evidence to be presented below.

Although the average percent crossing over for each of the regions studied (even for region 1 which does not include the waxy locus) is high compared with the average values from the Maize Linkage Summary (EMERSON, BEADLE and FRASER 1935), the observed values for each region fall within the range reported by other investigators. These values indicate that the allele responsible for the mosaic phenotype is at or as near to the waxy locus as can be determined by this method.

2. Segregation ratios

The data on segregation of wx^m in crosses involving $Wx\ wx^m$ and $wx^m\ wx$ heterozygotes appear complex at first sight. As shown in table 3, the ratios deviate greatly from those expected. However, the occurrence of prefertilization mutations producing non-mosaic kernels would lead to deviations of precisely the type observed. Type-crosses 1, 2 and 3 show that Wx is dominant to wx^m; the excess of non-waxy kernels in type-cross 3 is considered to be due to pre-fertilization mutations of wx^m to Wx' (Wx by mutation). In type-crosses 4, 5 and 7, where only mosaic kernels are expected, non-waxy and waxy non-mosaic kernels are also found, again presumably as the result of pre-fertilization mutation. Type-crosses 4, 5 and 6 show that wx^m is dominant to wx.

That the mosaicism is due to somatic mutations can only be inferred, since no germinal cells are involved in endosperm development. But pre-fertilization mutations can be tested directly by back-crossing to check for the presence of a stable allele on the chromosome which previously had carried wx^m.

TABLE 3

Dominance and segregation of wx^m.

Type-cross	Phenotype of F_1 endosperm					
	Non-waxy	Mosaic	Waxy	% non-waxy	% mosaic	% waxy
1- *Wx Wx* × wx^m wx^m	12 ears*	....	....	100	0	0
2- wx^m wx^m × *Wx Wx*	22 ears	....	....	100	0	0
3- *Wx* wx^m selfed	2618*	408*	3*	....	....	....
	1308	340	19	....	....	....
	3926	748	22	83.5	15.6	0.5
4- wx^m wx^m × *wx wx*	556	2391	74	18.4	79.2	2.4
5- *wx wx* × wx^m wx^m	1385	2057	131	38.8	57.6	3.6
6- wx^m *wx* selfed	2798	3970	2420	30.5	43.1	26.4
7- wx^m wx^m selfed	693*	2024*	19*	....	....	....
	1048	2266	81	....	....	....
	1741	4290	100	28.2	70.0	1.6

* Data of EMERSON and DEMEREC.

The demonstration that the non-mosaic kernels found on these ears give rise only to non-mosaics in succeeding generations would constitute evidence for the mutation hypothesis.

3. *Mutation to stable non-waxy (Wx′)*

Non-waxy F_1 kernels from type-crosses 4, 6 and 7 were grown and either selfed or back-crossed by *wx wx*. Of the 64 ears involved in this test, 83 percent indicated by the F_2 segregation that a *Wx′* allele, and not wx^m, had been inherited. Kernels from eleven of these F_2 families, each tracing back to a different mutation to *Wx′*, were grown, selfed and back-crossed for a third generation. In all cases, the resulting ears were completely non-mosaic (either all non-waxy or segregating for non-waxy and waxy). This constitutes evidence that the unexpected non-waxy kernels resulted from pre-fertilization mutations from wx^m to a stable *Wx′*, inherited by both endosperm and zygote.

In the 17 percent remaining cases, the endosperm was non-waxy but the zygote remained wx^m. A similar lack of correspondence between endosperm and zygote was found in 459 mosaic kernels, 13.8 percent of which proved, in subsequent crosses, to have non-waxy (*Wx′*) embryos. Apparently, then, the occurrence of noncorrespondence between endosperm and zygote is not specific to the non-waxy kernels, but is a general phenomenon in this material.

Two well-known processes may contribute to the non-correspondence: hetero-fertilization, and the occurrence of mutations in the gametophyte. When a plant carrying the wx^m allele is used as pollen parent, heterofertilization (SPRAGUE 1932) may occur, since mutations from wx^m to *Wx′* occur during tassel development. Thus, pollen from a plant homozygous for wx^m may be effectively heterozygous for this locus.

Mutations occurring in the female gametophyte can be scored directly without the complicating factor of hetero-fertilization, by using F_1 kernels

from type-cross 4, $wx^m\ wx^m \times wx\ wx$. The non-waxy and mosaic classes of these F_1 kernels were grown separately and selfed or back-crossed to determine the F_1 genotype of the zygotes. The total amount of non-correspondence between endosperm and zygote was found to be 8.4 percent for 202 F_1 plants, including both types: (1) zygote wx^m but endosperm non-waxy, and (2) zygote Wx' but endosperm mosaic.

The writer has unpublished data suggesting that under certain conditions a wx^m allele may mask the presence of a mutated non-waxy allele in the endosperm, thus providing another source of non-correspondence. However, the fact that most non-mosaic non-waxy kernels breed true, and remain stable in subsequent generations, has been taken as critical evidence of germinal mutation from wx^m to Wx'.

4. *Mutation to stable waxy* (wx')

It is difficult to determine definitely whether mutation to a stable recessive allele has occurred. In stocks characterized by very late and few mutations, kernels may appear totally waxy while still carrying a wx^m allele.

In an effort to isolate mutations of wx^m to wx', every waxy non-mosaic kernel from ears of type-crosses 4, 5 and 7 found during the first two growing seasons of this study, was grown to check the F_1 zygote for the presence of a stable recessive allele. In addition, a random selection of waxy kernels from similar crosses made in later generations were also tested. In all, 415 F_1 waxy kernels selected from over 28,000 kernels examined, were planted and of 160 F_2 ears recovered, all but two were segregating for mosaic kernels.

Of these two cases, one segregated for wx^m in the F_3 and the other appeared too recently for study.

These data indicate that stable germinal mutations to wx' occur with an exceedingly low frequency, if at all.

5. *Tests for chromosomal aberration*

Mosaicism of the type observed might be ascribed to aberrant chromosomal behavior leading to unequal crossing-over and position effect, or to breakage.

Plants carrying the wx^m allele were crossed to KYS, an inbred line notable for the cytological excellence of its pachytene chromosomes. Observations were made on the meiotic divisions of chromosomes from microsporocytes of several F_1 plants, in the search for aberrant behavior. In particular, the short arm of chromosome 9 was examined closely; nothing abnormal was observed. During subsequent studies of heterozygous wx^m material, further cytological observations of meiosis were made and again no abnormalities were seen. LAUGHNAN (1949) has recently shown that the mutation of A^b to A^d in maize is correlated with the occurrence of crossing-over.

A re-arranged chromosome 9 * was used to test whether the appearance of waxy mosaicism was correlated with the occurrence of crossing over. This

* The writer is indebted to DR. B. MCCLINTOCK who suggested this test and provided suitable re-arranged chromosome 9 stocks.

chromosome, recovered by McCLINTOCK from seed fertilized with X-rayed pollen, was greatly re-arranged as the result of multiple breaks and rejoinings, so that crossing over between it and a normal 9 occurred in only about one percent of reduction divisions in the region c-sh-wx (McCLINTOCK 1941). Plants homozygous for *wx* and for re-arranged 9 were crossed reciprocally with plants homozygous for wx^m and for normal 9. The F_1 kernels were mostly mosaics of the familiar types, and the rest were non-waxy kernels, due to pre-fertilization mutation. Thus the expression of mutability was unaffected by the absence of meiotic crossing over.

It seemed possible that the wx^m mosaicism might be due to chromosome breakage of the type reported by McCLINTOCK (1946–47) in which an unstable allele, *Ds* (dissociation) mutates in such a way that the chromosome carrying this allele breaks at the dissociation locus, under the influence of another unstable locus, *Ac* (activator). The possibility that breakage might be occurring in the wx^m material, was tested with the following crosses:

1. $$\frac{c\ sh\ wx}{c\ sh\ wx} \times \frac{C\ Sh\ wx^m}{C\ Sh\ wx^m}$$

2. $$\frac{C\ sh\ bz\ wx}{C\ sh\ bz\ wx} \times \frac{C\ Sh\ Bz\ wx^m}{C\ Sh\ Bz\ wx^m}$$

In the first case, if a chromosome break were associated with production of waxy-mosaics, the areas on the kernel mosaic for waxy, should also be mosaic for shrunken and colored aleurone. In the second cross, a chromosome break would produce bronze aleurone spots corresponding to the non-waxy spots. Except for a few colorless spots (occurring with the low frequency normally found), neither shrunken nor aleurone color mosaics appeared, in striking contrast to the waxy mosaicism.

More recently, McCLINTOCK (1947–48) has reported many other mutable loci, including two waxy mutables, in stocks carrying *Ac*. She states: "Because many of the mutations occurring at the two mutable *c* loci, the wx^{m-1} locus, and the mutable *Ac* locus do not result in dicentric chromosomes, as do *Ds* mutations, and do not lead to detectable gross chromosomal aberrations, the mechanism, if it is a breakage phenomenon, must restore the normal chromosome morphology. Unequal breakage within the locus, followed by the criss-cross type of fusion mechanism, could accomplish this end." If such a mechanism is involved in the wx^m material, it would not have been detected by the methods used.

However, it should be pointed out that the appearance of the pollen in the two mutable waxy stocks, wx^{m-1} and wx^{m-2}, described by McCLINTOCK, differs markedly from that of wx^m stocks. In the case of wx^{m-1} only 2–3 percent of the pollen was blue-staining, and in the wx^{m-2} material, less than half of one percent. Of the blue-staining pollen, in the first case about 50 percent were defective grains, small and only partly filled with starch; and in the second case, almost all the blue-staining pollen was defective.

In the wx^m material, on the other hand, high percentages of normal size, blue-staining pollen grains were found, even in late mutating lines, as pointed out above. In crosses of *c wx/c wx* by *C* wx^m*/c wx*, equal numbers of colored and colorless kernels were found, indicating no appreciable deficiency attributable to wx^m pollen.

6. *The addition of heterochromatin*

To determine the effect of additional heterochromatin on mutability, the wx^m allele was crossed into a *wx wx* stock containing several *B* chromosomes (heterochromatic chromosomes found in some maize stocks and presumed to be genetically inert, RANDOLPH 1941), and heterozygous for abnormal 10, a special form of chromosome 10 which contains a long extra heterochromatic segment (LONGLEY 1937). There were no apparent effects from this additional chromatin on the mosaic phenotype of F_2 kernels from plants carrying abnormal 10 as well as 2, 3 or 4 *B* chromosomes.

From the data considered in this section, it appears that the mosaicism is due to mutation of an unstable allele at the waxy locus, and that the instability is an intrinsic property of this allele. The direction of pre-fertilization mutation is primarily to a stable dominant allele. No mutations to a stable recessive allele were found.

PHENOTYPES OF THE MUTATED ALLELES

In considering the mutation hypothesis in the preceding section, the absence of endosperm mosaicism was taken as the main criterion of stability, and non-mosaic kernels were classified into two groups: red-staining with iodine and blue-staining with iodine. It was noted that no fully stable mutants were recovered from the red-staining group, but a large number of stable mutants were found of the blue-staining type. All the latter group, collectively referred to as *Wx′*, were dominant to wx^m and *wx*, and grossly indistinguishable from the dominant non-waxy (*Wx*) of field populations.

The question may now be raised: are these members of the *Wx′* group all reversions to the standard non-waxy allele, or have new intermediate alleles arisen by mutation from wx^m? In the case of a_1 mutating under the influence of *Dt* (RHOADES 1941b), several new intermediate alleles were found. They were characterized by clear differences in the intensity and distribution of anthocyanin pigments. The absence of any such gross differences among the *Wx′* group made necessary the use of a sensitive method in the attempt to distinguish small phenotypic differences.

Of the phenotypic differences summarized in table 1, the composition of endosperm starch is most accessible to quantitative study. That it is under the control of the waxy locus is shown by the data of SPRAGUE, BRIMHALL and HIXON (1943) and BRIMHALL, SPRAGUE and SASS (1945) summarized in table 4. Another factor to be considered is the narrow range of variability in percent amylose which has been reported in assays of many different varie-

TABLE 4

The effect of waxy allelic dosages upon endosperm starch composition.

(from HIXON and BRIMHALL, 1944)

Corn	Genotype of endosperm	Percent amylose
1. Iowa 939 (1943)	*Wx Wx Wx*	28.7
1 × 2	*Wx Wx wx*	28.7
2 × 1	*Wx wx wx*	23
2. Iowax 1	*wx wx wx*	0
3. Iowa 939 (1944)	*Wx Wx Wx*	27.7
3 × 4	*Wx Wx wx-a*	27.4
4 × 3	*Wx wx-a wx-a*	26.7
4. Argentine waxy	*wx-a wx-a wx-a*	2.4
5. Argentine waxy	*wx-a wx-a wx-a*	2.4
5 × 6	*wx-a wx-a wx*	1.3
6 × 5	*wx-a wx wx*	0.65
6. Iowax 1	*wx wx wx*	0

ties, hybrids, and inbred lines of the constitution *Wx Wx Wx*, summarized in table 5. These data indicate the small effect on this character attributable to the combined action of environmental fluctuations and genetic modifiers.

In view of this narrow range of variation and of the availability of excellent methods, percent amylose in endosperm starch was adopted as the phenotypic criterion by which to assess the *Wx'* group of back-mutants. Any heritable new amylose-amylopectin ratio found, which was significantly outside the normal range, would be considered evidence of a new allele.

The values reported in the literature for percent amylose were determined by potentiometric titration with iodine (BATES, FRENCH and RUNDLE 1943). In this study percent amylose was determined both by titration and by the blue value method of MCCREADY and HASSID (1943).

Both methods are based on the differences in iodine binding by amylose and amylopectin, which in turn derive from the structural differences between them. The amylose fraction consists of long straight chains of glucose residues in α-1,4 glucosidic linkage, and the amylopectin fraction of branched chain molecules in which the branch points are formed by α-1,6 linkages (HASSID 1943; BATES, FRENCH and RUNDLE 1943; SCHOCH 1945).

Amylose molecules assume a helical configuration in the starch-iodine complex (RUNDLE, FOSTER and BALDWIN 1944; RUNDLE 1947). The color of the complex depends upon the number of iodine molecules inside the helix, and this in turn depends on chain length. A straight chain of at least 20 glucose residues is required before any iodine is bound. As chain length increases and more iodine molecules are bound, the complex becomes colored, and the color gradually shifts from reddish-brown to purple and finally to the intense deep blue characteristic of amylose.

Amylopectin also binds iodine, but much more weakly than does amylose. The color of the amylopectin-iodine complex is reddish brown to purple, depending upon specific conditions.

Starch forms complexes with many substances as a result of helix formation. Of these, fatty acids are of particular importance, since they are present in the cell and presumably in the leucoplast in which endosperm starch is deposited (WHISTLER and HILBERT 1944). Fatty acids can and do interfere with starch-iodine complex formation (SCHOCH and WILLIAMS 1944). Intensive defatting is therefore necessary before determining the percent amylose by any method based on complex formation.

TABLE 5

*Percent amylose in Wx Wx Wx corn from different sources.**

Corn	Percent amylose†	Source
Iowax 939 (1943)	28.7	HIXON and BRIMHALL 1944
Iowax 939 (1944)	27.7	" " " "
Iowa I 205	28.5	MACMASTERS, private comm.
Iowa O. S. 426	28.5	" " "
Golden Cross Bantam sweet	28.6	" " "
"Cheesy" endosperm	26.9	" " "
U. S. 13	29.5	" " "
Os 420	28.5	" " "
Sutton's White Dent	28.9	" " "
Champion White Pearl	30.5	" " "
Cuzco, Peru—Yellow	29.5	" " "
Cuzco, Peru—White	28.6	" " "
Commercial dent hybrids	27.3–28.3	T. J. SCHOCH, private comm.
S-78	28.9	Present investigation
S-123	28.3	" "
S-92 (brittle)	27.8	" "
Commercial corn starch	27.8	" "

Mean—28.49 Standard deviation—0.78

*WHISTLER and WEATHERWAX (1948) assayed 39 maize varieties from North, South and Central America for amylose content. They reported values ranging from 23.8% to 30.3% amylose. These data have not been included in table 5 since they were obtained by a micromethod with an error of at least 5%.

MACMASTERS has recently informed the writer that several hundred corn samples have been assayed at the NORTHERN REGIONAL RESEARCH LABORATORY for percent amylose, and they found 21–23% amylose to be "not uncommon in starch of corns of foreign origin." Also, a number of corn samples were found to contain 30–55% amylose. Although no information was provided about the origin of the high amylose samples, it is known that attempts are being made to breed commercial varieties of high amylose corn, using the recessive genes *su* and *du* shown by CAMERON (1947) to modify the amylose-amylopectin ratio. No other modifiers have been reported as yet.

†Computed from iodine affinity data on the basis of iodine affinity of crystalline amylose of 18.7 mg. I_2/mg. amylose (BATES, FRENCH and RUNDLE 1943).

The colorimetric method of MCCREADY and HASSID is based on the fact that the amylose-iodine complex follows Beer's Law at 640μ, under standardized conditions. The method is a direct one for determining amylose concentration, since amylopectin present in the solution does not contribute appreciably to light absorption at 640μ, nor does it interfere in the determination.

The potentiometric titration method is based on the fact that under certain standardized conditions, starch-iodine complex formation removes iodine

from solution in a quantitatively reproducible manner; and that the amount of iodine in solution at any stage in the titration can be determined potentiometrically. Since both amylose and amylopectin bind iodine, the percent amylose is computed from the experimental data by an empirical method.

Thus, the blue value and potentiometric methods are based upon entirely different measurements. The amount of iodine bound by amylose is determined directly in the blue value method, by the intensity of color of the complex produced. In iodine titration, on the other hand, the EMF measurements depend upon the free iodine in the solution, which is not bound by the starch. Since the methods are so different and are subject to different sources of error, they can be used to check each other. The fact that both methods are empirical makes the use of comparative checks especially important.

Starch samples were prepared according to a procedure suggested in part by DR. T. J. SCHOCH. Seventy-five kernel samples were steeped 48 hours in 0.45 percent sodium bisulfite at 50°C. Embryos were dissected out, the endosperms ground in a Waring Blendor and filtered through nylon bolting cloth. The filtrate, containing suspended starch grains, was incubated over night with 2 mg of trypsin at pH 8.0, sedimented from water and then from methanol until the supernatant was clear and the starch grains observed under the microscope were free from protein. At this time the samples were also checked for red-staining starch grains, the presence of which would indicate instability of *Wx'*. The starch was then rescreened through bolting cloth with methanol and dried. The samples were pasted with boiling water and disintegrated in a Waring Blendor (semi-micro container) for ten minutes, precipitated with more than four times the volume of methanol (in some cases 95 percent ethanol was used) and allowed to settle until the supernatant was clear. The supernatant was then decanted, the precipitated gelatinized starch was washed with 95 percent ethanol and transferred to a modified Soxhlet extractor. All samples were defatted 30–36 hours with 95 percent ethanol.

Titrations were run with a Leeds and Northrup automatic recording potentiometer, which had been adjusted to record EMF values within the range of 0.2 to 0.3 volts. Iodine was added automatically during the titration from a motor-driven hypodermic syringe connected through a gear-box to the paper feed of the potentiometer. The data were recorded automatically in the form of graphs of the titration with EMF plotted against distance on the paper (convertible to ml I_2 added).

Forty back mutants to be assayed for percent amylose were selected at random. Each mutant traces back to a non-waxy kernel on a different ear derived from selfed or back-crossed $wx^m\ wx^m$ or $wx^m\ wx$ parents. The non-waxy kernels were grown and selfed in most cases for two generations, in eleven cases for three generations, to check on the stability of the mutated allele, and to establish homozygosity. Assays were run on homozygous *Wx' Wx' Wx'* kernels.

The results are shown in table 6. Not a single new amylose-amylopectin ratio appeared among the 40 back mutants assayed. The mean value of 28.2

TABLE 6

Percent amylose in endosperm starch of 40 back-mutants.

Family	Iodine titration values		Blue values
	Iodine affinity (mg I_2/mg starch)	% amylose*	% amylose
161	5.05	27.0	27.6
162	5.5	29.4	29.7
165	4.9	26.2	28.3
167	5.2	27.8	28.0
169	5.2	27.8	29.1
166	4.9	26.2	27.4
17	5.1	27.3	29.4
152	5.5	29.4	27.3
153	5.55	29.7	31.8
158	5.1	27.3	28.9
172	5.2	27.8	28.3
298	5.55	29.7	26.9
303	4.8	25.7	27.4
322	5.1	27.3	29.6
338	5.2	27.8	29.1
443	5.0	26.8	28.3
444	5.3	28.4	30.3
448	5.7	30.5	30.9
449	5.4	28.9	29.1
459	5.4	28.9	27.7
465	4.8	25.7	26.9
522	5.1	27.3	28.2
556	5.75	30.8	30.4
558	5.2	27.8	30.3
560	5.4	28.9	30.6
561	5.4	28.9	27.6
563	5.3	28.4	28.6
565	5.3	28.4	29.6
566	5.45	29.1	31.0
567	5.1	27.3	
568	5.06	27.1	28.3
569	5.55	29.7	29.4
571	5.5	29.4	29.6
573	5.4	28.9	30.6
574	5.3	28.4	28.9
575	5.3	28.4	29.1
576	5.3	28.4	28.3
577	5.1	27.3	28.4
578	5.6	30.0	30.0
580	5.4	28.9	29.1
Mean	5.44	28.22	28.97
Standard deviation	....	1.32	1.30

* Computed from iodine affinity on basis of iodine affinity of crystalline amylose, 18.7 mg I_2/mg amylose. (BATES, FRENCH, and RUNDLE 1943).

percent amylose for those samples determined by iodine titration is to be compared with the mean of 28.5 percent for corn from field populations as shown in table 5. It is apparent that the values in tables 5 and 6 represent samples from the same "population" in the statistical sense. The error of the titration method was found to be 2 percent on the basis of duplicate values, run

for the most part on different days. This compares well with values of 1 percent and 2 percent reported by various investigators.

The mean of 28.97 percent amylose determined by the blue value method is higher than the mean of 28.22 percent determined by iodine titration. However, the standard deviations are practically the same in both cases, 1.30 and 1.32, indicating a similar range detected by both methods. From these data one cannot say which set of values is more accurate, since both methods are empirical. The important observation is the comparability of values, which demonstrates the relative accuracy of the results. Also the closeness of check of iodine titration and blue values is a further evidence of the validity of both methods.

On the assumption that some of the mutants might show differences when heterozygous but not when homozygous, ten of the 40 mutants were also assayed with the *Wx'* allele heterozygous. In these cases, *Wx' Wx'* plants were crossed reciprocally with a *wx wx* inbred line, and the endosperm starches of *Wx' wx wx* and of *Wx' Wx' wx* kernels were assayed and compared.

TABLE 7

Percent amylose of heterozygotes compared with homozygotes.

Family	*Wx' wx wx*	*Wx' Wx' wx*	*Wx' Wx' Wx'*
465	21.4	27.1	26.9
561	20.0	26.5	27.6
563	20.6	....	28.6
566	22.6	28.0	31.0
569	21.4	....	29.4
571	21.9	28.2	29.6
573	21.9	26.5	29.6
574	21.4	28.0	28.9
577	21.9	27.4	28.4
578	20.0	28.0	30.0
Mean	21.2	27.5	29.0
Standard deviation	0.86	0.69	1.37

The results are shown in table 7, together with the *Wx' Wx' Wx'* assay values for these mutants taken from table 6. Both sets of values for the heterozygotes have very low standard deviations, indicating that all ten mutants carry the same allele, within the limits of error of the methods.

The average value of 21 percent for *Wx' wx wx* kernels indicates that the incomplete dominance described by SPRAGUE, BRIMHALL and HIXON for the *Wx* allele of their stocks, holds also for the back-mutated *Wx'* allele herein described.

In summary, no new alleles have been found to arise as the result of back-mutation from wx^m to the stable dominant form. The phenotypes of forty back-mutants carrying the *Wx'* allele were indistinguishable when homozygous from kernels carrying the dominant allele found in field populations. Ten of these mutants, assayed also as heterozygotes, showed the incom-

plete dominance characteristic of the waxy gene, but no significant heterogeneity within the mutant group.

MUTABILITY AS A PHENOTYPE

A striking characteristic of kernels carrying the wx^m allele, is the variety and complexity of mosaic patterns found. Two sources of this pattern variety are: (1) differences in time and frequency of mutation during endosperm development, and (2) the known irregularity of endosperm development (RANDOLPH 1936). Mutations occurring early in development would be expected to give rise to larger spots or sectors than those occurring late, and if mutations occurred at random times during development, the resulting kernels might show a great variety of patterns, even with a rather similar mutation frequency. The irregularity of endosperm development further complicates the subjective appraisal of mosaic patterns, although there is some evidence that the irregularity of early development is not very extensive and is randomly distributed (STADLER 1939).

Despite the apparent complexity, a study of mosaic pattern inheritance as a phenotypic expression of wx^m was undertaken. The problem was approached first by attempting to determine the time and direction (red-staining to blue-staining, or the reverse) of mutation in endosperm tissue as a basis for interpreting mosaic patterns, and then to select pattern types of heritable constancy.

1. *Direction of mutation*

A study was made of the extent of mutation at various stages of endosperm development in order to determine the direction of mutation, and also the relation of time of mutation to the final extent. The F_1 kernels examined came from crosses of $wx\ wx \times wx^m\ wx^m$, the wx^m parents being sib plants from Type 1 kernels (fig. 1 and below) which produced F_1 kernels mostly of Type 1. Ten-kernel samples were taken from each of nine developing ears at 2–3-day intervals, starting 14 days after pollination.

The fraction of blue-staining starch grains per endosperm at various stages of development was taken as a measure of the extent of mutation. The starch grain rather than the cell was considered as the unit, since endosperm cells contain either red-staining or blue-staining starch grains, but not mixtures of the two. Red-staining and blue-staining grains are essentially the same size and shape. The ratio of red-staining to blue-staining grains was determined for each endosperm (embryo removed) by crushing the cells, staining with iodine, and differentially counting a drop of suspended starch grains in a haemocytometer. Stable waxy and non-waxy kernels observed as controls stained uniformly red and deep-blue respectively at the time the first starch was laid down and throughout development.

The results are shown in figure 5, where each point on the curve represents the mean value for 20, 30 or 40 kernels, depending upon the number of ears sampled on a given day. Most kernels contained no blue-staining

starch grains, or less than one percent, at 16 days after pollination, the earliest stage at which starch was clearly evident. One kernel at this stage, and eight kernels at later stages of development, were completely blue-staining. It is assumed that these kernels represent pre-fertilization mutations to *Wx'* and consequently are not included in the mean values.

From the data of figure 5, it appears that the net direction of endosperm mutation is from red-staining to blue-staining starch.

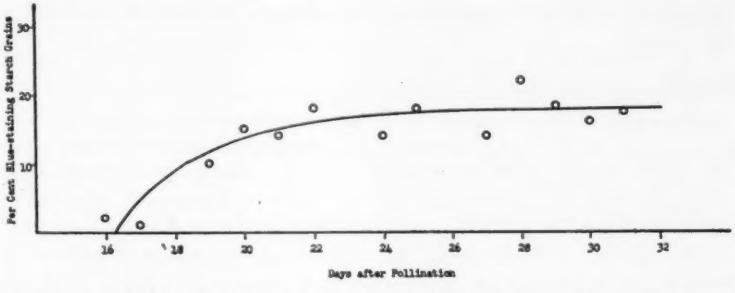


FIGURE 5.—Increase in blue-staining starch during development of mosaic kernels (*wx*^{m} *wx wx*).

2. *Mosaic pattern types*

On the evidence that the direction of endosperm mutation is from an allele responsible for red-staining to one for blue-staining starch, in Type 1 kernels, some inferences can be made about the mosaic patterns shown in figures 1–4. Kernels classified as Type 1 (fig. 1) contain small discrete spots of blue-staining tissue on a continuous red-staining background. From the small size of mutated areas, it is inferred that the mutations occurred (or were expressed) later than in the other pattern-types. Frequencies as low as one mutated spot per kernel have been observed.

Type 3 kernels (fig. 3) in which the mutated areas are predominant and continuous, surrounding isolated red-staining spots, look as if the direction of mutation were reversed in this material. However, when immature kernels of this type were assayed for percent blue-staining starch grains, the values were found to increase from 50–60 percent blue-staining at 16 days after pollination, to 70–90 percent blue-staining at 21 days. These data, though only from four ears, suggest that the direction of mutation is from red-staining to blue-staining in this material as well as in Type 1, and that the red-staining spots are residual un-mutated areas which have been surrounded in the course of endosperm development by the predominant blue-staining tissue.

Intermediate patterns of all sorts have been observed between the extreme Types 1 and 3. Of these, a common and easily recognized "network" pattern has been designated Type 2 (fig. 2). It is assumed that differences in time and frequency of mutation will account for the various intermediate patterns observed, but they have not yet been studied in detail.

Figure 4 shows four sectored kernels. In the first, the light-staining region appears to be Type 1 and the dark-staining region to be Type 3. The second kernel is sectored for Type 1 and a blue-staining non-mosaic region. In the third kernel, which resembles the second, the frequency of mutated spots is so high that the pattern approaches that of an intermediate type. The fourth kernel is sectored for Type 2 and an apparently waxy non-mosaic region. In most cases, only one combination of pattern-types has been found among the sectored kernels on a given ear, and that combination has been represented not only by half-and-half sectors, but also by sectors of other sizes and shapes.

3. *Inheritance of mosaic pattern*

In general, the attempt to select various patterns of heritable constancy was very difficult. Type 3 kernels have shown the most predictable pattern inheritance yet found in this material. Crosses were made involving several stocks, in which the wx^m parent was from a Type 3 kernel. Of the resulting ears, 31 were entirely non-waxy ($Wx\,Wx$ or $Wx\,wx$), 45 ears gave rise to ratios summarized in table 8, and one contained only intermediate type kernels.

TABLE 8

Inheritance of type 3 mosaics.

Type-cross	F_2 endosperm phenotype			% mosaic of wx^m class	
	Non-waxy	Mosaic (Type 3)	Waxy	Av.	Range
4. $wx^m\ wx^m \times wx\ wx$	323	213	0	40.0	14–59
4a. $wx^m\ wx \times wx\ wx$	543	146	747	21.2	3–39
6. $wx^m\ wx$ selfed	1165	309	453	20.9	2–68
7. $wx^m\ wx^m$ selfed	910	259	....	22.2	3–73

These data indicate that in most cases, Type 3 kernels give rise to two types of ears: (1) altogether non-waxy, and (2) segregating for a high and variable percent of non-waxy kernels, the rest being Type 3 mosaics. The ear with intermediate type kernels is unexplained; its occurrence suggests the possibility that mutation occurred from a factor for Type 3 mutability to one for intermediacy.

Kernels on ears resulting from selfed $wx^m\ wx$ Type 3 plants, were segregating for one, two, or three doses of the wx^m allele, but there were no apparent differences in pattern to correlate with dosage differences.

When Type 1 kernels were selected from ears containing various pattern-types, grown and selfed, most of the resulting ears were again mixtures of pattern-types. In a few cases ears were found either wholly Type 1 or segregating sharply for Types 1 and 2. On ears wholly Type 1, as in the case of Type 3, dosage differences (one, two or three wx^m alleles) had no apparent effect upon the mosaic pattern.

In both cases of selfed heterozygotes (Types 1 and 3), no markers were present to allow one to distinguish the wx^m dosage of individual kernels. However, on each ear the kernels showed very great uniformity of mosaic pattern, although they were segregating for one, two and three wx^m alleles. Thus it was clear that the mosaic pattern was not altered significantly by the dosage.

Preliminary crosses of Type 1 with Type 3 have given rise to F_1 kernels of intermediate pattern types only. However, in most cases, plants from intermediate patterned kernels do not produce ears segregating simply for Types 1 and 3 in the following generation.

From the investigation to date, it appears that in endosperm cells containing wx^m, the time and frequency of endosperm mutation is itself under hereditary control. This hypothesis is suggested by the heritability of Types 1 and 3, the sectored kernels segregating sharply for two pattern-types, and the intermediate patterns arising in F_1 kernels of Type 1/Type 3 crosses.

DISCUSSION

The purpose of this investigation was to study some aspects of gene mutability and gene action by analyzing in detail the behavior of a single locus. The waxy locus was chosen because there was available for study a strain of maize in which the endosperm starch was a mosaic mixture of cells with waxy and cells with non-waxy starch. This strain was of interest not only for the analysis of this mosaic character, but also because the expression of the waxy locus was amenable to biochemical study. The discussion will take up: (A) the mosaic phenomenon, and (B) mutation and gene action as interrelated characteristics of the waxy locus.

A. *The mosaic phenomenon*

1. Cytogenetic evidence

The mosaic phenotype described in this paper (figs. 1–4) has been shown to result from the existence of a highly mutable allele at the waxy locus. This conclusion is based upon two lines of evidence.

a) The principal features peculiar to plants carrying the wx^m allele are: endosperm tissue which is a mosaic mixture of waxy and non-waxy starches; and stable non-waxy plants arising from wx^m parents. Gametes carrying a stable dominant allele, Wx', have been recovered from plants which were $wx^m\ wx^m$. This constitutes direct evidence of mutation. It is inferred from this that the mosaic endosperm results from the same mutational process. However, this inference cannot be tested directly since endosperm tissue does not produce gametes.

b) No evidence has been found for chromosomal aberration in this material. The observed normality of meiosis and the absence of any evidence of chromosome breakage have indicated that no gross aberrations are involved. Meiotic crossing over is not a factor in the mutability since mosaicism and pre-fertilization mutations occur with the same frequencies in the absence of

crossing over due to a chromosome rearrangement. The mutability cannot be attributed to some other gene which induces a waxy allele to mutate, since the mosaic character is not lost by out-crossing and has been localized at the waxy locus by linkage analysis.

These data are consistent with the hypothesis that the mosaic phenotype results from mutation. Two classes of mutation are generally recognized: those which can be correlated with some aberrant chromosomal behavior or rearrangement, and those which cannot. McCLINTOCK (1950) has recently postulated a new mechanism to explain mosaicism and mutability in the maize stocks which she has been studying. This mechanism involves the insertion of a submicroscopic piece of heterochromatin into or near a given locus, inhibiting the action of the dominant allele present, and appearing consequently like a mutation to the recessive allele. In some cases, removal of the heterochromatic material restores the locus to full activity, and appears therefore like a back-mutation. Associated with this phenomenon are a series of events which include chromosome breakage and rejoining and further aberrations derived from these. McCLINTOCK reports that in some cases these events depend upon the presence of a special gene, *Ac* (activator), while other mutable loci require no activator. However, both groups show the same kinds of expression of variegation. In the cases described (McCLINTOCK 1947–48, 1948–49) these expressions included tissue mosaics due to losses of genetically marked sections of chromosome, pollen abortion, chromosome rearrangements, and the origin of mutability at many other loci.

With the available methods, we have found no evidence of any detectable aberrations in the wx^m material. Neither chromosome breakage, nor abnormal amounts of pollen abortion, nor rearrangements, nor the appearance of new mosaic characters, have been observed. Consequently, from our data, the mosaic-waxy phenomenon appears to be a case of "point mutation": mutation with which no other genetic or cytological event has been correlated. This conclusion is supported by the other findings of this study: the kind of stable alleles resulting from mutation of the unstable allele, wx^m; and the genetic control of mutation rate.

2. Alleles at the waxy locus

A primary characteristic of the waxy-mutable strain is the occurrence of large numbers of germinal mutations back to a non-mosaic stable condition. An analysis of the nature of these reversions was undertaken to try to find new alleles of waxy. It was assumed that the kinds of alleles arising by mutation from wx^m might throw light on the nature of the mutation process, and that new alleles, not found in nature, might appear. Such alleles would be of great interest both in connection with the mutability of wx^m and with studies on the biochemical action of the waxy locus.

The search for new alleles was abetted by the fact that RHOADES (1941a) had found several at the a_1 locus arising under the mutagenic action of *Dt*, FOGEL (1950) found them after mutation of an unstable allele at the *R* locus, and McCLINTOCK (1948–49) found them in her material at several loci.

(As noted earlier, in some mosaic kernels, staining with iodine revealed small cell clusters which stained various shades of lavender, in addition to the characteristic red- and deep blue-staining regions which predominated. It might be postulated that the lavender regions carried intermediate alleles. However, in the absence of any germinal mutations to lavender-staining, or even of endosperm sectors large enough for chemical analysis, it has not been possible to choose among a number of alternative hypotheses concerning the lavenders which have been proposed.)

Three alleles at the waxy locus have been found in nature. Several phenotypic differences have been correlated with them (table 1). Of these differences, the percent of amylose in endosperm starch was employed in the present investigation. At present, two types of starch molecules are known, amylose and amylopectin. Amylose is a linear polymer of glucose residues in α-1,4 linkage; amylopectin is a branched glucose polymer in which the straight-chain linkages are α-1,4 and the branched points are α-1,6 (MEYER 1942; HASSID 1943; SCHOCH 1945). SPRAGUE, BRIMHALL and HIXON (1943) first showed that the ratio of amylose to amylopectin molecules in endosperm starch varied with the waxy alleles present. On the basis of this and subsequent investigations (summarized in tables 4 and 5), it was considered that this amylose-amylopectin ratio was a reliable criterion for the identification of new alleles of waxy.

Accordingly, forty independent back-mutants from wx^m to a stable non-waxy condition, selected at random, were assayed for percent amylose of endosperm starch. No significant variation in percent amylose was observed among these forty mutants (table 6). The range was 25.7 percent to 30.8 percent amylose (standard deviation 1.32) comparing favorably with the range and mean values reported in the literature for non-waxy starches from field corn (table 5). Consequently, it was concluded that in all forty cases, mutations had occurred from wx^m back to the same non-waxy allele, and that it was indistinguishable from the non-waxy allele of field populations.

The absence of new alleles arising from wx^m recalls the behavior of the waxy locus in nature. Although waxy has been in constant use by geneticists as a convenient and popular marker locus during the last 40 years, only three stable alleles have been found (Wx, wx, and wx^a). Altogether, less than ten independent mutations from dominant to recessive have been found (summarized in HIXON and BRIMHALL 1944), and wx^a was discovered only recently in Argentina (ANDRES 1944).

In recent years a concerted search for maize starch with unusual amylose-amylopectin ratios has led to the assay of non-waxy ($Wx\,Wx\,Wx$) endosperm starch from a large number of varieties and inbred lines, and has revealed no intermediate alleles of waxy among these stocks (table 5). The absence of any alleles but Wx among the back-mutants studied in this investigation is therefore consistent with the allelic non-diversity of this locus in nature.

Consequently, the absence of any alleles but Wx arising from wx^m adds further support to the hypothesis, already indicated by the cytogenetic data,

that the mutation process involved in the mutable waxy phenomenon is no different in kind from the process of spontaneous mutation occurring in "stable" stocks. What is drastically different in the mutable-waxy material is the very high mutation rate.

3. Mutation rate and pattern inheritance

The mutations of wx^m, which result in mosaic endosperm and stable back-mutants in the germ line, do not occur at random. The time and frequency of these mutations is under gene control, as shown by the inheritance of mosaics of Type 1 (low mutability, fig. 1), and Type 3 (high mutability, fig. 3). In some selected families of Type 1, the mutability is so low that the kernels have very few tiny spots, sometimes only one. These stocks closely approach the appearance of stable wx lines. This similarity suggests that the difference between stable and unstable alleles is basically a function of mutation rate control.

From this point of view, the mutability of wx^m no longer appears as a problem of the waxy locus, as it did at the outset of this inquiry. From the cytogenetic evidence, and from the identity of the reverted alleles with those found in nature, the mutability of wx^m appears no different from the spontaneous reversion of wx to Wx, except for its rate of occurrence. Consequently, the question of how the mutation rates of these stocks are genetically controlled appears to the writer as a focal center for further investigation.

B. *Interrelations between mutation and gene action*

We turn now to a related question: can one explain the paucity of alleles at the waxy locus, as observed both in field populations and in this study?

From the genetic point of view, this paucity of alleles is not in accord with the common occurrence of multiple alleles at other loci. Intermediate alleles of waxy would also be expected for biochemical reasons. As shown by SPRAGUE, BRIMHALL and HIXON (1943), the waxy allelic dosages determine the molecular composition of endosperm starch. Waxy ($wx\ wx\ wx$) endosperm starch contains no amylose, $wx^a\ wx^a\ wx^a$ has 2.4 percent, and $Wx\ Wx\ Wx$ has about 28 percent (table 4). From these data, intermediate alleles corresponding to intermediate amylose concentrations (between 2.4 and 28 percent) would appear likely. Nonetheless, no such alleles have been found either in nature (table 5) or after back-mutation from wx^m (table 6). (MCCLINTOCK [1948–49] has reported intermediate waxy alleles. Four of them were assayed by the writer, who found two were in the range of Wx, and two were similar to wx^a.)

An hypothesis has been developed to account for these observations, which brings together the known facts about the waxy locus and may be useful as a basis for further investigation. It is suggested that the paucity of waxy alleles may be a result of the action of the waxy locus in endosperm development, at least in part, rather than being ascribable entirely to changes in the gene itself.

1. Biochemical action of the waxy locus

The waxy locus has been viewed as a factor in starch biosynthesis since the work of BRINK (1928, 1929a, b; BRINK and ABEGG 1926). This view has been greatly strengthened by recent studies (SPRAGUE, BRIMHALL and HIXON 1943) made possible by progress in the field of starch chemistry (especially BATES, FRENCH and RUNDLE 1943). SPRAGUE, BRIMHALL and HIXON have shown that the ratio of amylose to amylopectin in endosperm starch is a phenotypic expression of the waxy locus. The question then arises: can one determine how the amylose-amylopectin ratio is controlled?

Phosphorylase is the enzyme responsible for α-1,4 linkages (CORI and CORI 1936, 1937; HANES 1940a, b); under suitable conditions, amylose molecules can be synthesized in vitro from glucose-1-phosphate with phosphorylase. The synthesis of amylopectin is less well understood. Presumably, phosphorylase is responsible for the α-1,4 linkages, but several preparations have been proposed as catalysts for branch formation (CORI, SWANSON and CORI 1945; BOURNE and PEAT 1945; BOURNE, MACEY and PEAT 1945; BARKER, BOURNE, WILKINSON and PEAT 1950; BERNFELD and MEUTEMEDIAN 1948a, b).

These authors all suggest that the structure of amylopectin is determined by the ratio of phosphorylase to branching enzyme activity, despite unclarities about the mechanism of amylopectin synthesis. Recently, BOURNE and collaborators have shown experimentally that branched chain starches with different properties result from in vitro synthesis of polysaccharide from glucose-1-phosphate with enzyme mixtures containing different proportions of phosphorylase and "Q" (BARKER, BOURNE, PEAT and WILKINSON 1950; BARKER, BOURNE and WILKINSON 1950). This work greatly strengthens the view that the ratio of activities of phosphorylase to the branching enzyme controls the structural properties of the amylopectin molecule.

The difference between waxy and non-waxy starch results from changes in the ratio of amylose to amylopectin molecules. No structural difference between the amylopectin of waxy starch and the amylopectin fraction of non-waxy starch has been detected although many comparative studies have been made (T. J. SCHOCH, private comm.). Consequently, it has been assumed by the writer that the ratio of phosphorylase to branching enzyme activities in waxy and non-waxy endosperms is very similar, since the amylopectins are so similar, and that the ratio of amylose molecules to amylopectin must be attributed to a different system.

How then can one interpret a single gene difference leading presumably to a single chemical difference, which determines the presence or absence of 28 percent unbranched amylose molecules in the starch? One interpretation of these allelic differences from a chemical viewpoint, is that tissues producing amylose contain a substance, a branching inhibitor, which prevents a constant proportion of the developing glucose polymers from becoming branched. This substance might act by adsorption or perhaps by specific reaction with the number-6 carbon atoms, making them unavailable to the branching enzyme.

In any case, the starch formed in the presence of this branching inhibitor would contain some unbranched molecules, in contrast to waxy starch and to glycogen in which all the molecules are branched.

On this hypothesis, the amylose-amylopectin ratio would be controlled by the availability of the branching inhibitor, which might then be considered a direct or indirect product of waxy gene action.

One approach to identifying the branching inhibitor is suggested by the existence of maize and pea varieties with very high amylose contents. It was shown by CAMERON (1947) that in endosperm starch of supersugary maize (*su du Wx*), the amylose content is about 65 percent. Varieties of wrinkled peas are known in which the starch contains about 98 percent amylose. In both cases the total starch content is very low, suggesting a connection between high amylose and some block in starch synthesis. However, not all genetic blocks in starch synthesis have the same effect on starch composition. The writer assayed starch from brittle (*bt*) maize in which very little total starch was formed, and found its amylose content to be 27.8 percent, indicating no apparent effect on the amylose-amylopectin ratio in this case. Nonetheless high amylose varieties may be good material in which to look for substances controlling the amylose-amylopectin ratio.

When more sensitive methods are found to distinguish branched from unbranched short-chain glucose polymers, it should become possible to approach the problem of waxy gene action more directly. At present, however, some information about the action of the waxy locus can be inferred from indirect evidence. Genetic observations on allelism and mutation rate, considered together with physiological data on phenotypic variation, support the hypothesis that the paucity of waxy alleles results from the metabolic action of the waxy locus in starch formation.

2. Allelism and gene action

A relationship between alleles and biochemical action is shown in the curves of figure 6. In these curves the waxy alleles have been plotted against their phenotypic expressions, following the method of STERN (1929) and MULLER (1947–48). The critical problem in plotting these points is the spacing of alleles along the X-axis. Although there is a basis for an ordering of alleles (genetic dominance and increase of percent amylose), one cannot quantify the differences between them, since neither the basic unit of gene action nor the number of such units per allele is known.

To bring this out two hypothetical curves have been drawn in figure 6, which differ in the positioning of one point, d' (genotype $Wx\ wx\ wx$). The resulting curves embody very different relationships between alleles and phenotype. On the solid curve, the phenotype changes very rapidly at first with small increments of gene activity and then levels off so that further increments of gene activity have little effect. Thus there is only a small sector on the X-axis over which small changes in x are coupled with observable phenotypic changes on the Y-axis. In the dotted curve, on the other hand,

small increases of phenotypic effect result from small increments in gene activity over a very large range of values along the X-axis.

The solid curve may be regarded as the more likely representative of the waxy locus. This curve rises steeply between points *c* ($wx^a\ wx^a\ wx^a$) and *d* (*Wx wx wx*). This means that a small increment in gene activity in this range would yield a sharp increase in percent amylose. Consequently, the probability of finding any intermediate points between *c* and *d* would be very low indeed. Thus this curve is consistent with the observed absence of intermediate waxy alleles in nature and in this study.

To bring out the interrelationships under consideration, the behavior of the waxy locus may be compared with the *R* locus in maize, which is concerned with anthocyanin pigmentation. A very large number of alleles have

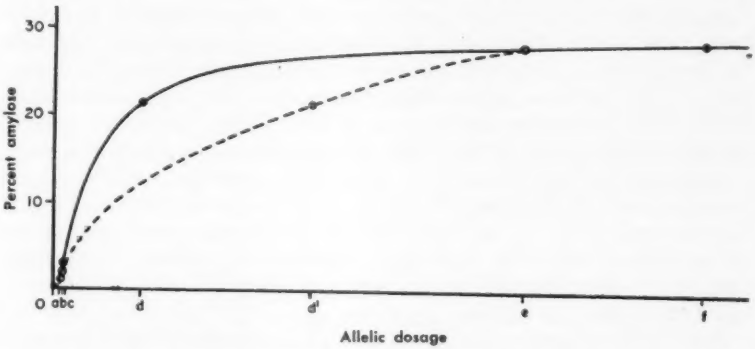


FIGURE 6.—A postulated gene-action curve for the waxy locus.

0—*wx wx wx*	d—*Wx wx wx* (in one location on the x-axis)
a—$wx^a\ wx\ wx$	d'—*Wx wx wx* (in another location)
b—$wx^a\ wx^a\ wx$	e—*Wx Wx wx*
c—$wx^a\ wx^a\ wx^a$	f—*Wx Wx Wx*

been described at the *R* locus (STADLER and FOGEL 1943, 1945), which correlate with small differences in the intensity and distribution of pigment over a large range. The dotted curve in figure 6 is suggested as a representation in broad outline of the behavior of the *R* locus.

Further support for this hypothesis comes from physiological data. The percent amylose of endosperm starch is an extremely stable characteristic. Maize varieties collected from North, Central and South America, differing enormously in genetic makeup, have shown great constancy in percent amylose (table 5). Similarly, maize grown under a great diversity of environmental conditions also shows this constancy. These facts reflect a biochemical system which is apparently quite insensitive to gross changes in cellular metabolism of the growing plant, except for the effect of supersugary (CAMERON 1947) noted above.

Various biochemical mechanisms could account for this insensitivity. In the simplest case, the solid curve of figure 6 may be considered as a plot of enzyme concentration against starch composition. On this curve, a very small amount of active enzyme would saturate the system, which levels off rapidly at 28 percent amylose. In such a system there is very little opportunity for the occurrence of fluctuations in percent amylose.

In contrast to the behavior of the waxy locus, the *R* locus may also be considered in this way. The phenotypes of the *R* alleles are markedly altered by environmental fluctuations, modifier genes and small effects of the residual genotype as well. These facts are consistent in a general way with a relationship between enzyme activity and phenotype which follows the dotted curve of figure 6.

An hypothesis has been proposed: that the rarity of alleles at the waxy locus can be explained in terms of the metabolic action of the locus. Although the specific action of the waxy locus is not known, the hypothesis is supported by the available indirect data. These data have been summarized in the form of a gene-action curve, from which it appears that the waxy locus has an exceedingly limited variability of expression. Only within the range of zero to about 5 percent amylose is there much opportunity for the appearance of new alleles, the occurrence of observable mutations, or the possibility of phenotypic variation. Above that range, the biochemical reaction which the waxy gene controls is apparently saturated very rapidly. Other gene changes may occur, but they remain phenotypically undetectable. The phenotype acts, in effect, as a filter.

The correlation between phenotypic and genetic variability which has been made in this study suggests certain limitations on the detection of alleles at other loci as well. In part, detection depends upon the sensitivity of observation methods. In part, however, mutations may be obscured by what we have called the phenotypic filter. Mutations which lead to changes in gene action either below the threshold of expression, or above the saturation point, will go undetected because the phenotype has, in fact, not been altered. With present methods, one cannot penetrate this phenotypic filter. Nonetheless, by assembling enough data on a given locus to construct a gene-action curve, one can attempt to assess the effectiveness of such a filter.

SUMMARY

1. The waxy mosaic phenotype is characterized by the occurrence of kernels mosaic for waxy and non-waxy starches, and by the appearance of an unexpected non-waxy class of kernels. The non-waxy kernels have been shown to result from mutations occurring before fertilization, of an unstable allele, wx^m, at the waxy locus, to a dominant non-waxy allele. The mosaic kernels result from mutations occurring during endosperm development, from wx^m to Wx.

2. Instability of the wx^m allele could not be ascribed to the influence of any other gene.

3. Instability of the wx^m allele could not be accounted for by any gross chromosomal aberration or correlated with meiotic crossing over.

4. A large number of stable pre-fertilization mutations to a dominant non-waxy allele were recovered; no stable recessive mutants were found.

5. Forty independent back-mutants from wx^m to a stable non-waxy allele (Wx') were assayed for percent amylose and found to approximate closely the range found in field populations. No new intermediate alleles were found in this randomly selected group of 40 mutants.

6. Ten of the back-mutants were compared as $Wx'\ wx\ wx$ and as $Wx'\ Wx'\ wx$ heterozygotes, with their homozygous sibs ($Wx'\ Wx'\ Wx'$), with respect to percent amylose. As a group the mutants showed incomplete dominance similar to that of Wx alleles from field populations, and such close similarity that all are considered to carry the same allele.

7. It was determined that the blue value method of McCready and Hassid for percent amylose gives results fully comparable with the potentiometric titration method, for corn starches in the range 20–30 percent amylose.

8. The time and frequency of mutation of wx^m are genetically controlled; both high and low mutability strains have been established.

9. A connection has been suggested between the lack of intermediate alleles in field populations of maize, the absence of intermediate alleles in this study, and the insensitivity of the phenotype to differences in residual genotype and environmental fluctuation. These data, summarized in a gene-action curve, suggest that the observation of multiple alleles at the waxy locus has been obscured by the nature of the biochemical reaction in which the waxy gene acts. It has been postulated that the waxy-gene-product may participate in an insensitive reaction which acts as a phenotypic filter, through which a large proportion of gene changes, if they occur, cannot, at present, be detected.

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ABSTRACTS OF PAPERS PRESENTED AT THE 1951 MEETINGS OF THE GENETICS SOCIETY OF AMERICA

MINNEAPOLIS, MINNESOTA, SEPTEMBER, 10-12, 1951

ABPLANALP, HANS and L.L. KOSIN, State College of Washington, Pullman, Washington. Heritability of growth in turkeys. — Estimates of heritability, based on combination of variance components (intra-class correlations) and intra-sire offspring-dam regressions, were made for progressively increasing body weights and for weight gains in growing Broad Breasted Bronze and Beltsville Small White turkeys. Several years' observations are involved. — Body weight was determined at four, eight, fourteen, and twenty-six weeks of age, while gains were calculated between the consecutive measurements. Both methods of estimation indicated similar trends in heritability: For males of both varieties it was low at four and twenty-six weeks, and high at eight and fourteen weeks of age; females showed highest heritability at four weeks. From the relative size of variance components it can be inferred that maternal influence and/or dominance effects must be high at early ages and in the last growth period. Sex linkage is indicated in Broad Breasted Bronze. — Negative offspring parent regressions in the first and last gain periods suggest the great importance of yearly changes in environment for those critical phases of development. There existed distinct differences in response to these factors, between sexes as well as between varieties. — Heritability estimates based on gains rather than actual weights do not seem to give increased precision in the evaluation of the genotype for growth in turkeys.

BERNIER, PAUL E., Oregon State College, Corvallis, Oregon. Micromelia VII, an overdominant recessive lethal in the domestic fowl. — A new lethal condition resulting in a marked reduction of limbs and skeleton observed in a family of S.C. White Leghorns. Chicks cannot hatch because legs cannot be used to break egg shell. Chicks are still alive within the shell on the twenty-second day of incubation with yolk fully absorbed. Down is rough and stringy like in ariboflavinosis. Body is covered with a slimy, albumen-like liquid. This new lethal condition is recessive as judged from the segregation ratios. The family in which the condition was first observed produced twenty percent more eggs than non-carrier families. This observation has led to the hypothesis of overdominance which is currently tested in a flock of eight hundred layers.

BRAUN, W. and ROBERT J. GOODLOW, Camp Detrick, Frederick, Maryland. The role of amino acids in bacterial population changes. — Earlier work with Brucella had demonstrated that the accumulation of alanine as a metabolite of most smooth strains growing under static conditions exerts an inhibitory effect on the growth of smooth cells and thereby creates an environment selectively favoring the establishment of usually nonsmooth mutants with increased alanine resistance. Recently obtained results extending this information on qualitative and quantative aspects of the effects of amino acids on population changes will be reviewed. Such results include the role of cultural conditions, especially the stereoisomeric configuration of the N-source employed; the effects of metabolites other than alanine; the effects of penicillin and antisera on modifications of metabolism and population changes; the lack of mutagenic effects of the selectively active amino acids; the role of differences between mutants in rate of metabolite-production and - resistance leading to population equilibria; and information on the mode of action of alanine. Data obtained with bacteria other than Brucella will be presented to indicate the general aspects of these phenomena. The bearing of these results on problems of modified virulence, resistance, and susceptibility, as exemplified by recent population studies with Salmonella in vivo, will be shown. Certain parallels between these phenomena and such problems as antigenic transformation (Paramecium) and differentiation will be discussed.

BREWBAKER, J.L., Cornell University, Ithaca, N.Y. Oppositional allelism in diploid and tetraploid alsike clover. — Nineteen self-incompatible (SI) F_1 plants of alsike clover were crossed in all combinations and reciprocally backcrossed with their SI parents. The F_1 plants segregated into 4 intra-sterile, inter-fertile groups of 5, 5, 6, and 3 plants, respectively, and all plants were reciprocally fertile with both parents. This behavior is best explained by the Nicotiana-type oppositional allelic system, in which the parents differ in both alleles. — Tetraploid sectors were produced on the parent plants following colchicine treatment, and tetraploids were also obtained from treating F_1 seedlings. The six genotypes derived in this manner were balanced diallelics. One parental and the four F_1 genotypes were self-incompatible, whereas the other parent was highly self-compatible (SC) in contrast to the SI diploid from which it was obtained. All selfed progeny of the SC parent were self-compatible, whereas the hybrid progeny from crosses to unrelated SI tetraploids segregated into 2:1 ratios of SC:SI plants. Likewise, SC plants were recovered among the F_2 families derived from intercrossing the SI-F_1 diallelics. Comparable data have been obtained in white clover. — All results are explained on the basis of the "competitive interaction" of the S alleles in heterogenic pollen (S_1S_2 of the SC parent ($S_1S_1S_2S_2$) resulting in the failure of the personate inhibition of pollen-tube growth of these heterozygous gametes.

BRINGHURST, ROYCE S., *University of California, Los Angeles, Calif. Genetic analysis of chlorophyll deficiency in Melilotus alba x M. dentata hybrids with some observations on meiotic irregularities. — Melilotus alba x M. dentata hybrid seedlings are chlorophyll deficient, surviving for periods ranging to four weeks. Differences in survival are associated with seed size of the alba parent (pistillate). Such seedlings are reared by grafting upon sweetclover stocks. M. alba backcross seedlings (reciprocal matings) ranged continuously from chlorophyll-defective lethals similar to the interspecific hybrid to normal green. Near 50 per cent were seedling lethals. Three genes studied in progenies from two non-lethal alba backcross plants cumulatively conditioned chlorophyll reduction in heterozygous and homozygous combinations. One (ch_3) significantly reduced chlorophyll when heterozygous in the presence of "normal" alleles of ch_1 and ch_2. Plants homozygous for "defective" ch_1 and ch_2 in combination and ch_3 alone or heterozygous for all three genes in combination are seedling lethals. There is evidence for at least four other genes similarly effecting chlorophyll deficiency in other backcross progenies. All such genes presumably originated in M. dentata. Chlorophyll deficiency also occurred in the limited number of dentata backcross plants and their derivatives. Extremely low fertility of the interspecific hybrid plants could in part be associated with cytological irregularities in pollen mother cells. Included was evidence for a reciprocal translocation in pachytene figures from a pollensterile derivative of a dentata backcross.

BROWN, SPENCER W., University of California, Berkeley, Calif. Activity of the nucleolus-organizer of Luzula campestris in relation to internal nucleolar structure. — The 6 bivalents of L. campestris are identifiable at pachytene by their characteristic chromomere patterns; a heavy, terminal chromomere is constantly associated with the nucleolus and has thus been identified as the nucleolus-organizer. During pachytene and diakinesis granules originate from the organizer and migrate toward the center of the nucleolus. The granules sometimes remain attached to the organizer by clearly stainable filaments, or may occur along two tracks leading from each of the homologous organizers. When first formed, the granules seem to be either about equal to or about half the size of the organizer. The granules tend to cluster together in the central region of the nucleolus and to decrease in size which the consequent formation of increasingly wider clear zones around themselves. Large, vacuole-like regions, with clear contents and dark rims, are very frequently observed; these have presumably been produced by prior activity of clusters of granules. Thus the nucleolar contents may show considerable variation, sometimes within a single anther: 0 to numerous granules, with or without well-developed hyaline zones, 0 to several of the large, clear regions with dark rims, or

*Research conducted at University of Wisconsin supported by Wisconsin Alumni Research Foundation.

neither or both types of structure. Such extreme variation makes it seem likely that the activity of the organizer is influenced by minor physiological changes. In water mounts of the tissue of the ovary wall, the nucleolus-organizer has been identified in resting nuclei as a differentially swollen structure, quite distinct from the remainder of the nuclear contents. In fixed material, it is not so differentiated from the numerous other heterochromatic structures characteristically observed in this and other tissues.

BROWN, WILLIAM L. and PAUL C. MANGELSDORF, Department of Plant Breeding, Pioneer Hi-Bred Corn Company, Johnston, Iowa and Harvard University, Cambridge, Mass. The effects on yield and morphology of the addition of teosinte germ plasm to maize. — Nine cultures representing varying amounts of teosinte germ plasm operating on an isogenic corn background were each crossed with four inbred lines of corn belt maize. The resulting F_1 hybrids were tested for grain yield and were also analyzed for morphological differences within and between tester groups. The data which will be presented graphically and by actual specimens indicate the following: 1. Reductions in yield (below that of controls) are in almost direct proportion to the amount of teosinte germ plasm introduced. 2. The addition of teosinte to maize reduces the number of nodes in the inflorescence and the vegetative parts of the plant but at the same time increases internode lengths. 3. One of the better indicators of teosinte influence in maize is shown to be a ratio of ear length/kernels per row. 4. Differential segmental effects involving chromosomes 3,4, and 9 are indicated for at least seven characters.

BRYAN, C.R. and W.J. MILLER. University of Wisconsin, Madison, Wisconsin. Interaction between alleles to produce a hybrid substance. — Following backcrosses to Columba livia of the hybrids and selected backcross hybrids from the mating of Columba guinea and C. livia, a cellular antigen called C, specific to guinea, has been obtained in unit form. This C. substance by suitable matings has been obtained in both homozygous and heterozygous state. Homozygotes and heterozygotes can be differentiated serologically and by progeny tests. A contrasting substance known as C', peculiar to livia, has been demonstrated. C of guinea and C' of livia are presumably produced by an allelic gene or genes. By absorbing antisera against cells of the heterozygotes (CC') with the cells of the homozygotes (CC), reagents specific for identifying the C' substance are obtained. — Of 13 antisera produced against cells of the heterozygotes (CC), ten were completely depleted of antibodies when absorbed (a) with pooled cells of both parental species or (b) with the cells of homozygotes (CC) pooled with cells of livia. However, two antisera thus absorbed would react weakly at the absorbing dilution (1/60) with CC' cells and one antiserum so absorbed reacted strongly (titer of 1/240) with the CC' cells (20 birds), but not at all with CC cells (5 birds). No such reactions have been observed in a

few antisera to the cells of the homozygotes. — These antisera to the cells of the heterozygotes contained antibodies specific to an antigenic factor different from those found in either parental species or in the homozygotes. Presumably, this "hybrid substance" is the result of an interaction of allelic genes, but whether of alleles producing C and C', respectively, or of other allels on the same chromosomes, is an open question.

BUNKER, HELEN P. and B. R. SPEICHER, University of Maine, Orono, Maine. Inheritance of physiological activity in Habrobracon. — Different stocks of Habrobracon vary noticeably in activity, stock #33 being sluggish and pectinophorae more active. Using phototropism as a gauge of activity segregated groups of 210 males and 210 females from each stock, in lots of ten, were timed as they passed through a dark tube illuminated at the far end, the number emerging being tabulated at one minute intervals for eleven minutes. Wasps used were two days old and unfed; temperature was controlled. The average times for wasps emerging within the time limit were, for stock #33 females 5.53 minutes, males 6.41, and for pectinophorae females 2.97 minutes, males 3.44. Average times for the two stocks differ significantly within each sex. The times for daughters from reciprocal crosses averaged 2.73 when the mother was pectinophorae and 3.49 when the mother was stock #33. Both times differ significantly from that of stock #33 females but not from pectinophorae females. Also the difference between these two reciprocal crosses is significant and suggests cytoplasmic effects. A backcross of daughters from pectinophorae females by stock #33 males gave 300 tested daughters whose average time was 3.74 minutes. This differs significantly from both pure stocks but more closely resembles pectinophorae. These results indicate that the tendency towards increased activity is inherited and is genetically dominant. However there may also be a cytoplasmic effect carried through the egg.

BURDETTE, WALTER J., Louisiana State University School of Medicine, New Orleans, La. Administration of diethylstilbestrol to the tu-36a strain of Drosophila melanogaster. — In view of repeated demonstrations that the incidence and growth of certain types of mammalian tumors may be influenced by sex hormones, a study was made of the effect of stilbestrol on a strain of Drosophila which is known to bear tumors. An isogenic, tu-36a stock was used, and the experiments were carried out in an incubator at 25 ± 0.5°C. The flies were raised on medium containing 1 g. stilbestrol per 100 ml., and the tumor incidence was determined for both P_2 and F_1 adults. The average length of 50 larvae from experimental, P_2 cultures was only 55.9 per cent as great as that of a similar number from control cultures, the measurements being 1.9 mm. and 3.4 mm. respectively. There were 2 tumors (0.41 per cent) in 486 P_2 adults with treatment and 18 tumors (0.86 per cent) in 2089 of those without treatment (P = 0.30). The tumor

incidence in 1097 F_1 flies from treated parents was 0.46 per cent, and in 2793 F_1 flies from parents without treatment it was 0.72 per cent (P = 0.34). Lethal mutation rate, determined at the same time by the Muller-5 method, was 0.18 per cent with 1105 chromosomes tested in the treated group and 0.11 per cent with 1821 tested in the control group (P = 0.63). Therefore no evidence for either a change in tumor incidence or mutation rate was found following treatment of the tu-36a strain with stilbestrol, although it is possible that an effect on tumor incidence may have been obscured by the nutritional state of the larvae.

CALDECOTT, RICHARD S. and LUTHER SMITH, Agronomy Dept., The State College of Washington, Pullman, Washington. The influence of heat treatments on the injury and cytogenetic effects of X-rays on barley. — Dormant seeds of Himalaya barley were subjected to the following treatments: 1) Control; 2) X-radiation only (15,000r); 3)Heat only (ranging from 75°C. to 85°C. for 30 minutes in different tests); 4) X-radiation followed immediately by heat; 5) Heat followed immediately by X-radiation. — The effects of these treatments were measured by: 1) Injury (height of seedlings at 14 days); 2) Survival to maturity; 3) Frequencies of chromosomal aberrations in root-tip cells and in pollen mother cells of X_1 plants (those grown from the treated seeds); 4) Frequencies of seedling mutation in X_2 head progenies. — The heat treatments alone had no perceptible influence on the injury, chromosomal aberrations, or mutations. However, such heat treatments applied before the X-radiation significantly reduced the injury resulting from the irradiation. The effect on injury was more variable if the treatments were applied in the reverse order, that is, X-radiation followed byheat. In some of these comparisons the injury was more and in others less than that resulting from X-radiations alone. Chromosomal aberrations (as measured by chromatinic bridges at anaphase in root-tip cells and by interchanges at the first meiotic division in pollen mother cells were reduced by 30-50 per cent, regardless of whether the heat treatment was applied before or after the irradiation. On the other hand, the mutation frequency was increased from 30-50 per cent in the seeds given these combination heat and X-ray treatments. Thus, the same combination treatments resulted in: 1) A decrease in chromosomal aberrations, and 2) an increase in mutations — a clear lack of parallelism between these two effects of ionizing radiations.

CHASE, H. B. and P. F. FENTON, Brown University, Providence, R.I. The expression of obesity in yellow mice.* — The association of obesity with the dominant gene, A^y, for yellow coat color is well known, yellow littermates, as A^ya, being obese and non-yellow littermates, as aa, not being obese. It is also generally observed that with inbreeding

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obesity is much reduced or entirely absent in these yellow mice. To restore obesity it is a common practice to mate the non-obese inbred yellows to mice of another strain and thus to obtain F_1 obese yellows. Such a procedure results in obese yellow hybrids only when certain strains are used, and success varies with different laboratories. In our laboratory the use of a high fat diet (47.5%) from the time of weaning, consistently causes marked obesity in both inbred and hybrid yellow mice, the non-yellow littermates remaining normal as to weight. Fatty livers have consistently been observed in obese yellow mice. Several liver tumors have been noted. The commercial stock ration (Purina Laboratory Chow) which is used in maintaining a healthy breeding colony of mice does not generally permit the expression of obesity and fatty livers in these yellow mice. The capacity to become obese apparently is present in all mice with the A^Y gene but may require special feeding to be expressed.

CLARK, A.M. and C; J. MITCHELL, University of Delaware, Newark, Delaware. — Radiosensitivity of Haploids and Diploids of Habrobracon. — Haploids and diploids of Habrobracon were exposed to x-rays during various stages of pupal development. The ability to emerge from the cocoon was taken as the criterion of viability. D-sage-eclosion curves show that diploids are more resistant than haploids at all stages of pupal development. During the earlier stages, diploids are about three times as radioresistant as comparable haploids, while during the later stages, the differential radiosensitivity is not as great. For haploid pupae the increase in resistance is exponential with age. Younger diploid pupae increase in resistance at a rate identical to haploids, but older diploid pupae do not increase in resistance as rapidly so that the "survival" curves for haploids and diploids tend to approach each other in older pupae. Investigation of egg and adult stages is being done. These data indicate that a correlation exists between the genome number and radiosensitivity. They suggest that the deleterious action of radiation upon somatic tissues may be due to injury to the genetic mechanism.

CONGER, A. D. and L. M. FAIRCHILD, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. The induction of chromosomal aberrations by oxygen. — Treatment of dry pollen grains of the plant Tradescantia with oxygen (greater than 99.5% pure) produces chromosomal aberrations which can be observed at the pollen tube division on pollen culture slides. The aberrations produced are identical with those found after irradiation with X or gamma rays. Pollen treated with oxygen from two different cylinders (evacuated 5 times, gas flushed 5 times) and pollen merely flushed with oxygen, all had aberration frequencies equivalent to that produced by about 600r of X rays in air. Control pollen (not evacuated) and air pollen (evacuated 5 times, air flushed 5 times) had almost no aberrations. — Exposure of oxygen-treated pollen to but 2 r of gamma rays caused so many

chromosomal breaks that an analysis could be made of fragment frequency only. The fragment frequency was higher than oxygen alone, and roughly about what would be expected from 1000 r of gamma rays in air.

COOPER, D.C., University of Wisconsin, Madison, Wisconsin. The transfer of desoxyribose nucleic acid from the tapetum to the microsporocytes at the onset of meiosis. — Globules of desoxyribose nucleic acid form at the surface of the tapetal nuclei at that stage of anther development when the nuclei of the enclosed sporogenous tissue have reached the leptotene stage of meiosis. These bodies move to the inner surfaces of the cells, pass through the cell walls and collect in the intercellular spaces between the microspore mother cells. The centrally located nucleus of each microsporocyte gradually shifts its position and comes to lie in contact with the cell wall. The more or less paired chromonemata become somewhat polarized with their kinetochores adjacent to that portion of the nuclear membrane which is closely associated with the cell wall. The intercellular globules of desoxyribose nucleic acid collect in abundance opposite the regions where the microsporocyte nuclei and cell walls are in contact. These bodies become closely appressed to the wall and their contents pass through it into the adjacent nucleus and along the paired chromonemata bringing them into close association. The chromonemata become larger in diameter and take a deeper stain coincidental with the inward movement of this material. Shortly thereafter the nucleus within each microsporocyte resumes a central position and meiosis proceeds. Such elaboration and inward movement of nucleic acid has been followed in eight species of plants and its presence between the microsporocytes at the leptotene-zygotene stage of meiosis has been observed in another six species.

DI PAOLO, J.A., Northwestern University, Evanston, Ill., An attempt to induce mutations in Drosophila melanogaster with nucleic acid components. — Third instar male larvae were treated by injection into the area of the testis of the test substance dissolved in Holtfrater's saline. All solutions were 10^{-4} and consisted of adenine sulfate, guanine hydrochloride, thymine, uracil, cytosine, and thiouracil. For a control, male larvae were injected with Holtfrater's saline. All surviving males were subsequently mated to Muller-5 virgins and the F_2 was examined for sex-linked lethals. The results of the experiments are as follows: adenine sulfate, 1,090 chromosomes examined, 1 mutation found; guanine hydrochloride, 722 chromosomes, no mutations; uracil, 651 chromosomes, no mutation; thymine, 475 chromosomes, no mutation; cytosine, 215 chromosomes, no mutation; and thiouracil, 486 chromosomes, no mutation. It is concluded that the substances used are not mutagenic when applied by the method of these experiments.

EDMONDSON, MARGARET, Indiana University, Bloomington, Ind., Induction of sterility mutations in ultraviolet-treated polar cap cells of Drosophila. — The embryos treated by Meyer, reported on in the parallel paper, were studied for induced and spontaneous recessive mutations which resulted in sterility or a high degree of infertility, but no externally visible effect. Both -sterility and -sterility were were found, but these were seldom associated. The -sterility mutants were at least twice as frequent as those with -sterility. The combined frequency of steriles (including partial steriles of strong effect) approximated that of lethals in the same material. Controls and photoreactivated embryos produced fewer complete steriles relative to partial steriles than did the embryos treated with uv alone. This result would seem to indicate that a significant proportion of the complete sterility induced when there is a high mutation rate may be caused by mutation to partial sterility in two or more loci simultaneously. — All completely sterile mutants which had been found in this and previous uv-treated embryo experiments in this laboratory were cross-tested for possible allelism. None of the 21 -steriles showed allelism (as evidenced by non-appearance of either complete or partial sterility in the F_1), while among 38 -steriles two pairs of alleles were found. This would indicate that a larger number of different loci in the second chromosome of Drosophila can produce sterility without other visible effects. — Work supported in part by grant from U.S. Public Health Service.

FABERGÉ, A.C., University of Missouri, Columbia, Mo. Ultraviolet induced chromosome aberrations in Maize.* — A comparison has been made between the effects of 2537A. Ultraviolet radiation and of medium hard X-rays on the chromosomes of Zea mays endosperm. Pollen carrying four linked dominant markers, I, Sh, Bz, Wx, all affecting endosperm characters, was treated and used for pollinating plants recessive at these loci. By the detailed analysis of the mosaic patterns obtained, a method due to McClintock, several kinds of chromosome aberrations can be identified. Information obtained by this method is different from that given by standard cytological examination. All four markers are located in the short arm of the 9th chromosome, which represents only 2.7% of the pachytene length of the entire complement. Only aberrations involving this marked arm are identified. No aberrations are missed because of selection since even the complete absence of this entire arm is viable under these circumstances. Simple deficiencies can be recognized, and breakage-fusion-bridge cycles initiated at any point distal to Wx. With certain limitations, rings, dicentric translocations and inversions if accompanied by a breakage-fusion-bridge cycle can be identified. Ultraviolet radiation produces all of these aberrations in large numbers. One of the most common effects is the production of breakage-fusion-bridge cycles. The same is true of

*Work supported by a grant from the U.S. Public Health Service.

X-rays. At the time of writing this summary, no consistent differences between X-rays and ultraviolet have appeared, though the analysis of further data may reveal such differences. It is particularly desired to emphasize that these conclusions apply only to maize endosperm.

FARNSWORTH, M.W., University of Missouri, Columbia, Mo. Effects of the homozygous Minute-IV deficiency on the development of Drosophila melanogaster. — Homozygous M-4 embryos were obtained from an inbreed of M-4/+ heterozygotes. M-4/M-4 embryos die in the egg stage but cannot be distinguished from controls until after 10 hours of development. Major embryological anomalies are found in the midgut, foregut, and central nervous system. Although formation of the midgut proceeds normally, lengthening and coiling of this structure is greatly retarded. Fragility of the walls results in the release of yolk to the hemocoel. The midgut and body spaces are crowded with this material even in late stages, while in controls, such yolk is rapidly utilized. Therefore, it is concluded that yolk metabolism of M-4 homozygotes is impaired. Foregut abnormalities consist of incomplete head involution, aberrant formation of the cephalopharyngeal apparatus, and failure of frontal sac development. The initially normal central nervous system is later characterized by loss of a variable number of ventral ganglia, usually the fifth through seventh. Certain interganglionic connectives fail to develop and two to three pairs of ganglia remain posteriorly when condensation of the nervous system occurs. Other abnormalities consist of incomplete differentiation of somatic musculature and failure of the esophagus, heart, and fat bodies to develop. The observations are interpreted in terms of the hypothesis that all anomalies of the M-4 homozygotes can be traced to a generalized retardation in development occurring just before the time when such defects become visible. (supported by a National Research Council AEC Predoctoral Fellowship)

FORD, LEOLA E., Iowa State College, Ames, Iowa. Heterozygous male-fertility in Coleus. — In Coleus a dominant gene (L) is responsible for deep lobes in leaves and the recessive results in shallow lobes. All observed plants of the genotype Ll have been male sterile. (Rife, 1940). In the fall of 1949 a male fertile green deep lobed plant designated 50-1 was selfed as test for homozygosity. The resulting progeny included a number of shallow lobed plants. Further tests of male fertile deep lobed plants are being conducted.

FOSTER, M., University of Chicago, Chicago, Ill., Pigment-Forming abilities in guinea-pig skin. — The inherited pigment-forming systems in rodent skin offer rich possibilities for the analysis of gene-enzyme relations. Preliminary studies on skin homogenates from guinea-pig foetuses, involving manometric measurements of oxygen consumption as well as observations on pigments formed in the reaction vessels, indicate the presence in foetal skin of both tyrosinase (producing

tan, brown and black pigments) and a tryptophane-oxidizing enzyme (producing yellow pigment), as well as inhibitors of both enzymes. These factors affecting pigmentation were previously demonstrated in mouse skin. The results obtained with various guinea-pig phenotypes are presented. (Work supported by a postdoctorate research fellowship from the National Cancer Institute, U.S. Public Health Service)

FRASER, F.C. and T.D. FAINSTAT, McGill University, Montreal, Canada. Strain differences in the incidence of cleft palate in the offspring of pregnant mice treated with cortisone. — The injection of cortisone into pregnant mice often leads to the appearance of cleft palate in the offspring. The effect varies with dose of cortisone and gestational stage at which treatment is begun. There are well-defined differences between strains in the incidence of offspring with cleft palate following maternal treatment. Cleft palate (without harelip) has never been observed in the offspring of untreated mice. Results for the various strains used, and preliminary studies on inter-strain crosses will be reported.

GARDNER, Eldon J., Utah State Agricultural College, Logan, Utah. Genes producing a maternal effect in Drosophila melanogaster. — The tumorous head abnormality was shown to depend on a sex-linked gene (tu-1) and a third chromosome gene (tu-3). The tu-1 gene produced a maternal effect detected from the results of reciprocal crosses. Three "wild" laboratory stocks (Stephenville, Oregon R, and Crimea) were shown to carry alleles of tu-1 ($tu\text{-}1^S$, $tu\text{-}1^O$, and $tu\text{-}1^C$). Three other "wild" laboratory stocks (Woodbury, Florida 19, and Formosa) were found to carry modifiers of tu-3. Twenty-two other "wild" stocks, eleven laboratory mutant stocks, six tumor bearing stocks, and two field collections showed no genetic relationship with tumorous head. The maternal effect was influenced by temperature during a temperature effective period in the egg and early larval stages. The average penetrance was 79 percent at 25°C. The females showed a higher penetrance and expressivity than the males. Tumorous head flies were less viable than the controls. The greatest loss occurred during the early developmental period which coincided with the temperature effective period. Increased temperature during the critical period influenced the viability of tumorous head flies much more seriously than the controls. A maternal effect for viability as well as for the visible expression was detected. The gene (tu-1) is located near f in chromosome I. This gene was found to have no influence on the visible tumorous head expression or the viability of the flies in the absence of tu-3 at either 21°C. or 30°C.

GAULDEN, M.E., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Prologned visible change produced by heat in the chromatin of living grasshopper neuroblasts. — Grasshopper embryos were subjected to heat by immersing eggs for 6 minutes in

water at 47° C. Embryos were made into culture preparations and observations on the living neuroblasts were begun immediately. Heat causes an increase in the refractive index of chromatin, the chromatin of different mitotic stages showing varying degrees of increased refractivity. Highly refractive chromatin is slightly birefringent in polarized light, while untreated chromatin shows no birefringence. The increased refractivity of heat-treated chromatin persists through several cell generations after treatment, which suggests that the change may be of genetic significance. — Heat does not produce any detectable changes in the DNA content or in the degree of polymerization of DNA in nuclei as determined by photometric analysis of Feulgen and methyl-green preparations respectively. It is suggested that the increased refractivity of chromatin is caused by reversible heat denaturation of the protein moiety of chromatin.

GILES, NORMAN H., JR., ALVIN V. BEATTY, and HERBERT PARKES RILEY, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. The relation between the effects of temperature and of oxygen on the frequency of X-ray-induced chromosome aberrations in Tradescantia microspores. — Previous experiments of Sax and others have shown that the frequencies of X-ray-induced chromosome aberrations in Tradescantia microspores are greater at low than at high temperatures. The discovery of the effect of oxygen in increasing X-ray-induced aberration frequencies suggested that the greater solubility of this gas in water at low temperatures might be responsible for this temperature effect. To test this possibility inflorescences were irradiated in experiments in which the amount of dissolved oxygen was varied over a similar range in terms of volumes per cent in two different ways: (1) by using a constant percentage of O_2 (5% O_2 plus 95% helium) and making a series of exposures at several different temperatures, between 0.6° and 40° C., and (2) by using a constant temperature (27° C.) and varying the percentage of O_2 in the exposure chamber. Cytological analyses were made of chromosomal aberration types at the four- to five-day interval following irradiation. Exposures were also performed in the absence of oxygen (in pure nitrogen) at various temperatures. The experiments in O_2 demonstrate that the increased yield of aberrations at low temperatures cannot be attributed entirely to increased oxygen solubility. There is a considerable additional effect of temperature itself. In the absence of oxygen, the unexpected result was obtained that the effect of temperature is exactly opposite to that noted in the presence of oxygen - the yield of aberrations is considerably higher than at low temperatures. This stiuation is true not only for chromosome aberrations but also for all types of chromatid effects. The discovery of this reversal of the temperature effect in the absence of oxygen makes it unlikely that the effect of temperature is to be attributed solely to a modification of the behavior of broken chromosome ends during the recovery process. Rather, it seems probable that temperature exerts its effect, at least in the presence of

oxygen, on either the formation or effectiveness of some substance (or substances) produced in the cell by the radiation and responsible for chromosome breakage.

GOWEN, JOHN W., and JANICE STADLER, Iowa State College, Ames, Iowa, and PLOUGH, H.H., and HELEN N. MILLER, Amherst College, Amherst, Mass. On the chemical basis for typhoid resistance in mice. — During the past four years we have been investigating some 79 different mutants of Salmonella typhimurium. These mutants have lost their capacity to synthesize an essential metabolite. Virulence and active immunization tests of the mutants and their parent strains were made in balanced experiments on six inbred strains of mice. A mutation, on the average, reduced virulence of the mutant over that of the virulent parent line. Different mutations were characterized by different effects on virulence. The mutants acquired specific characteristics for virulence as well as for the biochemical requirements the mutation process altered. Theonine, phenylalanine, or methionine requiring mutants showed increased virulence. Adenine, hydroxyproline, arginine, isoleucine and leucine requiring mutants had less virulence than their non-mutated parents. The tests for acquired immunity showed a high negative correlation, -0.59, between the virulence of the mutant bacterial line and resistance to the virulent challenge dose of 11C. An adenine requiring mutant which had almost no virulence for mice likewise caused no increase in resistance of mice previously infected with it. Hydroxyproline and arginine mutants showed similar effects. Other mutants, as leucine and serine requiring, were low in virulence and high in their capacity to immunize. Different mutants to the same requirement do not always show the same virulence or immunizing capacity, i.e., histidine requiring mutants.

GREEN, M.M., University of California, Davis, California. The detection of mutant isoalleles at the vermilion locus in Drosophila melanogaster. — Wild type isoallels at the white and cubitus interruptus loci in D. melanogaster and mutant isoalleles at the bobbed locus in D. Hydei have been reported previously by other investigators (Muller, Timofeef-Ressovsky, Stern, Spencer). By utilizing suppressor mutations it is possible to detect isoallelism among mutants at the vermilion (v) locus in D. melanogaster. A number of v mutants of X-ray and spontaneous origin which phenotypically appear to be indistinguishable may be separated on the basis of their phenotypes in the presence of suppressor mutants. Two allelic vermilion suppressor mutants, one spontaneous, one X-ray in origin, were used. Among the v mutants tested, some are suppressed in the presence of either suppressor mutant, while other v mutants are not suppressed by either and manifest the vermilion phenotype in the presence of either suppressor. The phenotypic behavior of the v mutants in the presence of the suppressor mutants thereby may be utilized to separate v mutants otherwise apparently identical. A further phenotypic separation of

unsuppressed v mutants appears possible on the basis of the phenotype in heterozygotes of the genotype: suppressor-v,v (suppressed)/ suppressor-v,v (unsuppressed). Such heterozygotes manifest phenotypes intermediate between vermillion and wild type, with the degree of intermediacy depending upon the particular unsuppressed v mutant used.

HEIDENTHAL, GERTRUDE and LEONARD CLARK, Russell Sage College, Troy, N.Y. and Union College, Schenectady, N.Y. Survival rates of Halbrobracon eggs treated in first meiotic metaphase with low and high voltage x-radiation. — Studies were made on the survival of haploid eggs treated in first meiotic metaphase with low voltage (124 kv) and with high voltage (50 Mev) x-radiation. Dosage ranged from 100r to 1750r. Standard techniques previously developed by A.R. Whiting were used throughout. Since females were not mated in the first series of experiments, parthenogenetic development occurred among the surviving eggs up to larval stages when the observations were discontinued. The curves for low and high voltage are practically identical: survival following treatment with 400r was 54% for high voltage and 51% for low; at 1500r the viability was about 5% for both types of radiation. The curve for low voltage is essentially a confirmation of that found by A.R. Whiting. — Other studies were made of eggs treated with low voltage but laid by females which were mated following irradiation. Under these conditions it would be expected that if recessive lethals were present, viability should increase. Since the curves for eggs from mated and from unmated females are so very nearly alike, it is tentatively concluded that death of eggs is to be attributed to dominant lethals.

HERSKOWITZ, I.H., Louisiana State University School of Medicine, New Orleans, La. Mutation rate in D. melanogaster males treated with formaldehyde and 2,4-dinitrophenol.* — Although earlier work failed to demonstrate that 2,4-dinitrophenol (DNP) is mutagenic, experiments were undertaken to determine whether it is capable of influencing the mutative action of formaldehyde. The rate of recessive lethal mutations on the X chromosome in gametes of males raised from 48 hr. of age on food containing 0.13% formaldehyde and either 15 or 60 mg DNP per 50 cc medium was determined by the Muller-5 method. Up to 12 gametes were tested per male. There were 27 lethals in 880 spermatozoa tested from 87 males treated with the lower amount of DNP, including 2 lethals from each of 4 males. In 98 males treated with the larger amount of DNP there were 19 lethals in 1040 gametes tested, including 2 lethals from each of 5 males. Males grown in food containing 15 mg DNP, or 0.13% formaldehyde, or both, were mated to attached-X females and more than 8000 male offspring

*This project was supported by a grant from the National Cancer Institute, U.S. Public Health Service.

examined for visible mutations. Visibles were not any more frequent when DNP was present in addition to the formaldehyde than when only formaldehyde was administered. It is concluded from these results that DNP does not enhance the mutagenic potency of formaldehyde. The importance of determining the mutation rate in single individuals for the interpretation of the action of mutagens is discussed.

HOLLANDER, W.F., Yale University School of Medicine, New Haven, Conn. Recessive genes epistatic to dominants. — The well known F_2 ratio 9 : 3 : 4 has long demonstrated that a homozygous recessive gene may mask a non-allelic gene, either recessive or dominant. However, text-book discussions and glossaries do not specifically include this phenomenon in the category of epistasis. There has been a tendency to limit that term to dominant genes. But since masking is the main point, greater inclusiveness seems desirable. Therefore a revision of the definition of epistasis is proposed: "The masking of the phenotype of a mutant or non-standard gene by that of another, not an allele." Where chromosomal anomalies have phenotypic effects, the term "mutant gene" may include them, also. — Examples of recessive mutants epistatic over dominants are not infrequent: in general, albinism or white, over any other color mutant; in Drosophila, vestigial wing over beaded; in mice, hairless over wavy; and in pigeons, red plumage color over dominant patterns, as well as porcupine plumage over silky.

HUESTIS, R.R. and RUTH WILLOUGHBY, University of Oregon, Eugene, Oregon. Eye spotting in the deer mouse. — Peromyscus with eyes both of which look red because they lack pigment in the stroma of the iris and in the choroid and sclera have young with both eyes red, with one red and one black eye or with both eyes black. All these classes of young may, in turn, have young in all these classes but not in the same proportions, there being parent offspring correlation in the amount of eye pigment. — Twenty-nine of fifty mice selected at random from a group all of which had one red and one black eye, proved, upon low power examination, to have spotted eyes; light spots in the black eye or dark spots on the red eye or both. Black eyes were more often spotted than red ones and spots were more often small then large. The light spots in dark eyes tend to be located near the entrance of the optic nerve and central retinal artery, while the dark spots on red eyes tend to be near the ciliary body. The gradient of pigment deposition is, therefore, similar to that of scleral differentiation and perhaps causally connected with it.

HUNGATE, F.P. and T. MANNELL, Reed College, Portland, Ore. Sulfur-35 as a mutagenic agent. Radioactive sulfur was chosen as a desirable isotope to test for mutagenic properties. Sulfur is a part of the sulfhydryl group which is known to be essential to some enzymes. The possibility that genes and enzymes are similar suggests that sulfur

may be necessary in the functioning of some genes. Sulfur-35 is easy to use in the laboratory due to its weak beta particle and its convenient half-life of 87 days. — Neurospora crassa was grown on Fries Medium in which sulfur-35 as sulfate had been substituted for the normal sulfur. After growing the mold for varying periods of time, the conidiospores were suspended and plated according to the method of Tatum, Barratt, and Cutter (Science 1949). — When the radioactive sulfur was undiluted with non-radioactive sulfur, a recovery of more than 10% biochemical mutants was obtained even when the radiation absorbed by the mold was well below 10,000 reps. When non-radioactive sources of sulfur were also present, this rate was reduced. The fact that the presence of carrier sulfur reduces the mutation rate suggests that the mutations may be induced not only by the ionizing beta particle but also by the transmutation itself. — The mutants identified have in most part shown an inability to synthesize some amino acid. Those whose requirements have been identified include argininеless, methionineless, and leucineless, as well as some having more complex requirements.

JENKINS, J.A. and G. MACKINNEY, University of California, Berkeley, Calif. Inheritance of carotenoid differences in yellow-tangerine hybrids of the tomato. — Repeated studies of the yellow-red and the tangerine-red hybrids have demonstrated that the two mutants, yellow (rrTT) and tangerine (RRtt) differ from the common red tomato (RRTT) at two independent loci. Red tomatoes contain about 95% lycopene, 5 beta-carotene and traces of other carotenoids. The tangerine has about the same total carotenoid content and about the same amount of beta-carotene. However, the predominant pigment is prolycopene with appreciable quantities of zeta-carotene and poly-cis-psi-carotene. On the other hand, yellow tomatoes have a much lower total carotenoid content, which is largely beta-carotene with small amounts of lycopene. — Although the yellow and tangerine loci have been placed in different linkage groups, there has been no previous report on the yellow-tangerine hybrid. As expected, the F_1 (RrTt) is red and has a carotenoid content indistinguishable from homozygous red. The F_2 segregated: 50 red: 17 yellow: 24 tangerine, which fits a 12:3:4 ratio. Carotenoid analysis of the F_2 and selected F_3 progenies showed that the F_2 tangerines were of two types in the expected 3:1 ratio. The first was like the tangerine parent, the second, though visibly similar, had a distinctly different carotenoid content. This second type, the double recessive (rrtt), surprisingly enough, was more highly colored than the yellow. The predominant pigments were prolycopene and other lycopene isomers (no evidence of all-trans-lycopene) together with appreciable amounts of beta-carotene and phytofluene.

JOHNSON, B. LENNART, University of California, Los Angeles, Calif. Effect of other genes and of potassium nutrition on the expression of the lethal factor in stocks. — In stocks the inheritance of double- and single-flowered plants in a ratio of about 55:45 is governed

by a dominant factor for single, linked with a factor fully lethal to the pollen but only partially lethal to the eggs or zygotes carrying it. Singles which alone produce seed are heterozygous for both pairs of factors. Variation in percentage of doubles appears to be related to adversity of environment as well as to genetic differences. Three strains differing in percentage of doubles were selected in one variety of stocks. The growth rates of doubles and singles in each strain were tested in sand cultures with three levels of potassium. On height of plant and length and width of the 8th leaf taken weekly, the doubles were significantly larger than the singles. The strain selected for the highest percentage of doubles was significantly smaller than the other two in plant height and length of leaf for both singles and doubles, indicating that the size difference was governed by factors other than the lethal, and suggesting that these factors favor the elimination of singles. Significant differences due to K levels for doubles and singles combined appeared in leaf length and width. Although genetic differences are frequently observed in the relative leaf width of doubles as compared with singles, there were no such differences between selections in this case. There were, however, significant interactions of K level with doubles vs. singles on leaf width and plant height.

JONES, DONALD F., The Connecticut Agricultural Experiment Station, New Haven, Conn. The induction of cytoplasmic pollen sterility and the resotration of fertility in maize. — Three different sources of cytoplasmic pollen sterility have been used to convert many standard inbred lines to the pollen sterile condition by successive backcrosses with normal plants. The three sources behave essentially alike, with some differences in potency, and are similar in their behavior to the cytoplasmic steriles in other species. Pollen production ranges from complete abortion to complete fertility with little or no effect on other characters. Cytoplasmic steriles, with restored normal fertility, may be maintained in the homozygous condition. When these plants are crossed with other fertile lines, not carrying genes for pollen restoration, the condition of cytoplasmic sterility reappears in the second generation as a Mendelian recessive. Thus within the species the expression of this cytoplasmic character is completely under genic control and cytoplasmic sterility can be induced by crossing two normal appearing fertile lines.

KIMBALL, E., Clinton Experimental Farm, Clinton, Conn. Recessive black plumage in the fowl. — Pyle-zoned black fowls occur as segregants of wild type X extended black, and wild type X autosomal barring. Selective breeding produces an array ranging from complete pyle-zoning, at one extreme, to self-black, at the other. Representatives of phenotypic classes within the array all breed as carriers of the pyle-zoning potential. Variable expression of the pyle zone is attributed to differential polygenic complements. — The symbol e^{+} is proposed for a basic plumage pattern gene associated with (1) specific

black pigment deposition, and (2) characteristic restriction of the latter as sexually dimorphic pyle-zoning. Fowls of the genotype (e^+ e^+) are designated pyle-blacks. Major genes for stippling (Sg), autosomal barring (Bg), and penciling (Pg) are epistatic to e^+ black, but hypostatic to E black. Gene e^+ is a recessive allele of e (columbian restriction), and of E (extended black). Brown Leghorns, Golden Campines, and Partridge Rocks are genetically black fowls of the genotype (e^+ e^+), black in these instances being hypostatic to secondary pattern factors. A study of junglefowl and junglefowl-hybrid phenotypes suggests that (e^+ e^+) is characteristic for the genus Gallus. — Wild type (e^+) black in the fowl is comparable to recessive black of mammalian pelage; whereas, extended (E) black in the fowl is comparable to mammalian dominant black. Wild type (e^+) black is recommended as standard of reference in determining dominance of secondary plumage pattern factors.

KIMBALL, R.F. and NENITA GAITHER, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Modification of the action of X rays upon Paramecium aurelia. — A number of agents which previously have been shown to modify one or more effects of X irradiation have been tested on Paramecium aurelia. The mutagenic action of X rays in doses of one to six kiloroentgens was found to be reduced when the irradiation was performed in an atmosphere of nitrogen or in the presence of 0.7 M ethyl alcohol. On the other hand, 0.001 M sodium hydrosulfite, a reducing agent, had no influence on mutagenesis. No effect of the metabolic state was found since irradiation of either fed or starved paramecia, with or without incubation with 0.01 M sodium formate, produced identical amounts of mutation. Likewise the rapidity of multiplication of the cells after irradiation, as influenced by temperature, was without influence. Two nongenetic actions, division retardation and death within twenty-four hours, were studied using doses of 100 to 300 kiloroentgens. Both the amount of division retardation and of death were decreased by 0.7 M ethyl alcohol present during irradiation. Sodium hydrosulfite (0.001 M) clearly protected against the lethal action but had no influence upon division delay or, perhaps, enhanced it. Neither starvation nor incubation with formate was found to have any effect. Thus there is at least one agent which affects a variety of different kinds of radiation damage in Paramecium. It may be suggested that some step which is common to the various effects is involved. However, it is possible that some agents influence the steps leading to certain kins of damage only or else act more effectively upon one kind than the other.

KIRBY-SMITH, J.S., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Effects of infrared irradiation on the frequency of X- and gamma-ray-induced chromosomal aberrations in Tradescantia pollen tubes. — Tradescantia pollen has been treated with 250 kv X rays and 1.3 mev gamma rays in combination with near

infrared radiation in the 9500-30,000 Å region. The temperature of the material during infrared radiation has been closely controlled to eliminate the possibility of thermal effects. Measurements of the infrared absorption spectra of Tradescantia pollen show that, in the present experiments, an appreciable amount of infrared energy is reaching the nuclei. Typical near-infrared absorption curves will be presented. The chromosomal aberration frequency in the pollen tube division following irradiation has been studied. No significant difference in the aberration frequency, including interchanges, has been observed between material treated with X or gamma rays alone and material given near infrared radiation immediately before or after exposure to X rays or gamma rays.

KOESTER, M.L., Brookhaven National Laboratory, Upton, L.I, New York. The effect of continuous gamma radiation upon starch formation in Zea mays pollen*. — Mature pollen from corn plants was tested with 0.2 per cent solution of iodine potassium iodide to determine those grains which were not completely filled with starch and for this study classed as abnormal. Plants used in this experiment received from 0.09 r to 222.2 r per day throughout the growing season. A relationship was found between dosage received and percentage of abnormal pollen produced. Pollen from plants which received dosages up to 1.3 r per day showed no increase over the control in the percentage abnormal, but for dosages above 2.6 r per day there was observed a gradual increase in the percentage of abnormal pollen grains. The highest dosage studied gave the greatest percentage of abnormal pollen, 57.0 per cent. When the log of the dose was plotted against the percentage of abnormal pollen, the data were shown to fit a portion of a sigmoid curve.

LAUGHNAN, J.R., University of Illinois, Urbana, Ill. Reaction sequence in anthocyanin synthesis in maize. — The factors A_1, A_2 and Bz are complementary in the synthesis of anthocyanin pigment in maize. When anthocyanin synthesis is blocked individually by the recessive forms of these genes, brown pigments are produced alternatively. Since the phenotypes associated with these three recessives are different it should be possible to obtain information about the sequence of action of these factors in anthocyanin synthesis. Thus in the doubly blocked a_1a_1 a_2a_2 BzBz plant the expected phenotype is that characteristic of the recessive form of the prior acting gene, either A_1 or A_2 in this case. — Preliminary genetic studies indicate that the phenotype of the a_1a_1 a_2a_2 BzBz is indistinguishable from that of the a_1a_1 A_2A_2 BzBz plant, which on a conventional basis is interpreted to mean that A_1 acts prior to A_2 in the biosynthesis of anthocyanin. However, no straightforward interpretation is possible in the case of the other double recessive which has been tested; A_1A_1 a_2a_2 bzbz plants resemble neither A_1A_1 a_2a_2 BzBz nor A_1A_1 A_2A_2 bzbz

*Research carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

individuals but exhibit a phenotype not previously observed. This new phenotype is explained if it is argued that A_2 (or Bz) agent is capable of transforming not only an anthocyanin precursor but is involved also in a step in the shunt synthesis leading to brown pigments when anthocyanin synthesis is blocked by bz (or a_2). The possibility that one gene may be involved in more than one step where, however, there is a high probability of a close relation between the substrates would be better tested with Neurospora, for which shunt pathways are known also and in which the substrates involved may more easily be identified.

LEDERBERG, E.M., University of Wisconsin, Madison, Wisconsin. Lysogenicity in E. coli K-12. — Lysogenicity or latent virus was accidentally discovered when a sensitive indicator strain (W-518) was found, isolated as a UV mutant for an unrelated character. Mixed culture of W-518 and K-12 or its derivatives resulted in the weak lysis of W-518. The virus obtained from plaques, lambda, is carried by all other K-12 derivatives in a latent form and to which W-518 is uniquely sensitive. Stably lysogenic W-518 strains can be isolated from plaques of free virus or virus liberated by lysogenic cultures. A third phenotype (immune) which is lambda-resistant, non-lysogenic, and non-infectable occurs among the survivors of lysogenic cells treated with UV or of W-518 exposed either to lambda or a second virus, lambda-2 (probably a mutant of lambda). The latter virus was detected following UV treatment of a lysogenic culture, and attacks all classes except certain immune forms. Lambda-2 cannot be demonstrated as a latent virus in the resistant survivors. However, the maintenance of lambda is not affected by resistance to lambda-2. Strains sensitive to lambda are always sensitive to lambda-2; resistance to lambda-2 in these forms is always accompanied by resistance to lambda. Systematic examinations of lysogenics reveals a high incidence of resistance to lambda-2, which might have been accumulated either by a high spontaneous mutation rate of sensitive to resistant bacteria, or by selection of resistant bacteria by virus mutants. The progeny recovered from intercrosses among lysogenic, sensitive, and immune strains (all sensitive to lambda) consists only of parental forms. The two immunes (sensitive and resistant to lambda-2) are non-allelic.

LEFEVRE, GEORGE, JR., and F.J. RATTY, Jr., University of Utah, Salt Lake City, Utah. X-ray induced effects in the white-Notch region of wild-type and white-mottled-4 chromosomes of Drosophila melanogaster. — Canton-S + and w^{m4} males were exposed to similar X-ray doses of approximately 5000 r. The relative frequencies of viable white mutations, inviable white changes, notches, and simultaneous losses of both the white and Notch loci were determined. Examination of nearly 35,000 offspring disclosed that the incidence of white lethals was sensibly the same in the two series, likewise the Notches. However, viable whites were twice as numerous in w^{m4} as in Canton-S +, and white-Notch deletions about 16x more frequent in

w^{m4}. In each series the number of Notches was almost exactly equal to the sum of the number of viable white plus the white lethals. — The failure to find more white lethals in w^{m4} than in Canton-S +, even though the w-N deficiencies were enormously more frequent, implies that many of the viable whites induced in w^{m4} were actually deficiencies that would have been inviable if a loss of similar extent had been produced in a normal chromosome where the w^{+} locus is surrounded by euchromatin. A consistent interpretation of the results can be made on the assumption that all X-ray induced effects, viable and inviable, actually are the result of destruction of genetic material. When viable, the loss may consist of only one gene or less, and be cytologically undetectable. Longer euchromatic deficiencies will be almost invariably lethal. The Notch locus may well be more "important" than the w^{+} locus. Any loss in the former, however small, is homozygous inviable.

LEIN, P.S. and J. LEIN, Syracuse University, Syracuse, N. Y. Suppression of non-allelic acetate requiring mutants of Neurospora. — Two acetate requiring mutants of Neurospora crassa were isolated independently and designated S210 and S34. Of all the growth factors tested, these mutants responded only to acetate or fatty acids. Presumably, the latter substances were degraded to acetate, thus permitting growth. Attempts to cross the mutants directly were unsuccessful. They did, however, form heterocaryons with each other and these were crossed separately with S210 and S34. Isolation of wild type ascospores from these crosses demonstrated that these mutants were nonallelic. A culture of S34 in liquid minimal grew after eight days. A cross of this culture to wild type yielded segregations indicating the presence of a suppressor gene. This was confirmed by crossing wild type containing the suppressor to S34. When the same wild type was crossed to S210, the acetate requirement was again suppressed in those mutants containing the suppressor gene. The genetic data showed that the genes responsible for the acetate requirements of S210 and S34 and the suppressor gene were on the same chromosome. The finding that a gene can suppress non-allelic mutants in this case but not in a case previously reported by Houlahan and Mitchell (Proc. Nat. Acad. Sci. 33:223, 1947) is tentatively thought to be due to the fact that in the mutants described here, another pathway for acetate formation has become functional in the presence of the suppressor gene.

LEVITAN, M., Virginia Polytechnic Institute, Blacksburg, Va. Response of the chromosomal variability in Drosophila robusta to seasonal factors in a Southwest Virginia woods. — In Drosophila robusta collected in a woods near Blacksburg, Virginia the frequencies of the gene arrangements in the left arm of the X and the left arm of the second chromosome showed significant changes between the spring and fall of 1950. Directional changes also occurred in the X-right arm but were not statistically significant. In the spring of 1951 these

frequencies were found to have generally returned to their spring, 1950, values. In previous work with this species near St. Louis, Mo. and New York, New York cyclic seasonal changes appeared to be absent. The Blacksburg data indicate that D. robusta resembles D. pseudoobscura and D. funebris in that, in at least some localities, the chromosomal polymorphism is utilized partly in adaptation to seasonal variations in the environment.

LIEB, MARGARET, California Institute of Technology, Pasadena, California. Studies of a bacteriophage carried by Escherichia coli, strain K12. — A phage, lambda, is normally carried by cells of strain K12 of Escherichia coli; small doses of ultraviolet radiation induce lysis of K12 cultures and the release of large numbers of lambda particles. A substrain called Sens (W1294 from E. Lederberg) is non-lysogenic and, unlike the original K12, sensitive to lambda. When lambda is grown on Sens, the latent period is 38-40 minutes and the burst size 70-150. 10% to 100% of the cells of a Sens culture are lambda resistant, depending on cultural conditions. The proportion of resistants ($Sens^r$) can be varied at will and there is evidence that genetic factors are not responsible. Sens cultures do not exhibit varying sensitivity to phages of the T series. In cultures predominantly sensitive ($Sens^s$), the majority of $Sens^r$ cells are pre-lysogenic, i.e., after brief exposure to lambda they give rise to cultures indistinguishable from the original lysogenic K12. There are few such prelysogenic ($Sens^{rl}$) cells in predominantly $Sens^r$ cultures. Lambda adsorbs on $Sens^r$ cells but remains sensitive to anti-lambda serum. Small doses of ultraviolet radiation cause loss of a much greater proportion of the $sens^r$ cells than of $Sens^s$ cells in a culture, particularly in cultures which are chiefly $Sens^s$. An absolute increase in the number of $Sens^s$ cells after ultraviolet treatment suggests that $Sens^{rl}$ cells can be converted from pre-lysogenic cells into phage yielders before infection with lambda.

LINDEGREN, CARL C. and GERTRUDE LINDEGREN, Southern Illinois University, Carbondale, Ill. Multiplicity function of genes controlling carbohydrate fermentation in Saccharomyces. — The complex breeding stock of Saccharomyces which we have developed in our laboratory is the outcome of the interbreeding of four species of Saccharomyces: S. cerevisiae, S. carlsbergensis, S. chodati and S. microellipsoideus, none of which is capable of fermenting melezitose. The ability to ferment melezitose was introduced into the stock by a series of crosses with a culture designated Y-650 as S. cerevisiae. This organism was capable of fermenting galactose, sucrose, maltose, turanose and melezitose, but neither -methyl glucoside nor melibiose, and fermented raffinose slowly. — Genetical analysis of breeding stock has demonstrated that the differentiation of the progeny with regard to the ability to ferment maltose could be ascribed to the presence of a gene pair MA/ma. The N.R.R.L.-Y-650 culture carried an allele of MA

which also controls the fermentation of maltose and has been designated MA^{TSZ} since it controls the fermentation of turanose, melezitose, and sucrose in addition to maltose, in contrast to MA which is incapable of conferring ability to ferment either melezitose or sucrose. Widespread samplings have revealed that all maltose-positive cultures are capable of fermenting turanose and that no ma cultures are capable of fermenting turanose. MA, therefore, is designated MA^{T}; the superscript indicates that the ability to ferment turanose is associated with the MA^{T} gene. The allele from Y-650 is designated MA^{TSZ}; the superscript indicating that turanose, sucrose, and melezitose fermentation are controlled by this gene.

LUCE, W.M., University of Illinois, Urbana, Ill. Reduction in facet number in full-eyed (reverted Bar) Drosophila by X-rays. — Larvae of an inbred full-eyed (reverted to full from Bar) strain of Drosophila melanogaster, kept at 28 degrees C., were treated with X-ray dosages ranging from 1000 to 3000 r applied at the rate of 500 r per min. The corneas of the eyes of the imagoes which developed from the treated larvae were dissected off, mounted on slides, projected, and the facets counted. The X-rays produced a reduction in facet number. The rate of reduction was approximately 0.075 facets per r. The X-rays when applied before the larvae were 54 hours old (reckoned from the time when the larvae were laid as eggs) had no effect. Larvae treated at anytime when they were from 59 to 80 hours of age responded with essentially similar rates of reduction in facet number per r applied, with some evidence that the rate was less for the lowest dosage used (1000 r). — The X-rays prolonged larval development. The rate of prolongation, approximately 0.011 hrs. per r, appeared nearly constant for all X-ray applications within the age limits (38-80 hrs.) used in this experiment with the qualification that the 3000 r treatments had a slightly diminished effect on the rate of prolongation.

MACFARLANE, EILEEN W.E., A.A. KATZBERG and J;E. ALBERS, Institutum Divi Thomae, Cincinnati, Ohio. Degrees of Mitotic Poisoning in corneal cells. — The right corneas of mice were irrigated every hour for six hours with phenyl mercuric hydroxide (PMOH) 3.4×10^{-5}M to 1×10^{-3}M. The left cornea acted as control. Comparison of the proportions of mitotic stages showed, with increased concentration, an arrest first in telophase, then in prophase (PMOH 1.4×10^{-4}M), finally in metaphase in c-mitosis with PMOH 3.4×10^{-4}M and stronger. With PMOH 3.4×10^{-4}M and shorter treatment, arrest in prophase appeared after one hour with alternating phases of increase and depression of anaphase until the spindle was suppressed after 6 hours. In the early phase of spindle suppression an increase in both prophase and metaphase occurrs and the M/P ratio is unchanged. Using basic phenyl mercuric nitrate (PMN) on rat corneas 7.8×10^{-5}M was the threshold for suppression of spindle formation with 6 hours treatment. Half this concentration caused an increase in prophases after 6 hours and 3 hours

later an amitotic phase appeared. The cells had recovered to normal 18 hours after end of treatment. A 1% beef spleen extract, similarly applied, produced a marked arrest at metaphase. After the extract was applied for 6 hours in PMN 7.8×10^{-5}M, normal mitosis was present, but there was an arrest in telophase indicating a slight degree of poisoning which affected the spindle fibres.

MARKERT, C.L., University of Michigan, Ann Arbor, Mich. Comparative gene stability in Glomerella and Neurospora. — More than 100 auxotrophic mutants of Glomerella have been induced by the mutagenic radiations - ultraviolet light, X-rays, and fast neutrons. The specific nutritional requirements of 74 of these mutants have been determined by methods comparable to those commonly used with Neurospora. These data make possible (1) a comparison of the qualitative mutagenic effects of the three types of radiation, and (2) a comparison of the relative frequencies of the various kinds of radiation-induced auxotrophic mutants in Neurospora and Glomerella. — The data do not reveal any qualitative differences in the types of mutants induced by the three radiations used. However, ultraviolet irradiation appears to induce the most easily repaired type of genetic damage, since ultraviolet-induced mutants revert to wild type much more frequently than X-ray or neutron-induced mutants. — Since Glomerella and Neurospora are related fungi, it is not surprising that the commonly occurring mutants of Neurospora are also found in Glomerella and in similar relative proportions. Thus the genes which control homologous reactions in these two organisms appear to possess, in general, comparable stabilities. In contrast to Neurospora all auxotrophic mutants of Glomerella are visibly different from wild type on a complete medium. Two unusual types of Glomerella mutants are those requiring biotin and one which requires glutathione.

MERRELL, D.J., University of Minnesota, Minneapolis, Minn. Selective mating in laboratory populations of Drosophila. — On the basis of mating experiments, the changes in frequency of four sex-linked recessive genes (y, ct^6, ras^2, f) in populations of D. melanogaster were predicted. The predictions were tested by observation of the actual changes in laboratory populations. Each mutant was in competition with its wild type allele. The genes, y and ct, which reduced the mating success of the males to about 0.1 that of the wild type, were eliminated most rapidly. Males with ras mated 0.5 as frequently as wild type males while mating in the case of f was at random. Elimination of ras was slower than that of y and ct from the populations, but more rapid than that of f. Thus, the results in the populations parallel closely the results of the mating experiments. That selective mating is a major factor in the elimination of y, ct, and ras but not f can be demonstrated by an analysis of the frequency of the mutant genes in the males and females separately. If the mutant males mate less frequently than the wild type, the frequency of the mutation

(estimated by the Hardy-Weinberg formula) will appear to be lower in the females than in the males. Such an analysis of the data from the populations shows the frequency of f to be the same in both sexes, but the apparent frequency of y, ct and ras is always lower in the females than the males.

MEYER, HELEN U., Indiana University, Bloomington, Ind. Photoreactivation of ultraviolet mutagenesis in the polar cap of Drosophila. — Ultraviolet of predominantly 2537 Å (G.E. germicidal lamp) was administered to the polar cap of Drosophila embryos, after filtering out visible and non-mutagenic ultraviolet waves. Both treated embryos and controls were divided into two groups: one protected from non-mutagenic radiation below 5400 Å during and for 52 hours after treatment, the other illuminated by visible light (15-watt Westinghouse "Daylight" fluorescent tube, 2-3 cm. distant) during and for 1-1/2 hours following uv treatment. The illuminated group showed considerable recovery from the uv effects, as evidenced by increase in rate of survival of embryos, higher fertility after reaching maturity, and greater number of polar cap cells per fly which resulted in functional germ cells. The last conclusion was based on the smaller average size of groups of mutants of common origin, relative to the total number of tested offspring from a treated individual. — A genetic technique, newly designed by Muller, for scoring mutations in the second chromosome, facilitated this investigation and gave significant evidence that "photoreactivation" occurs in the chain of processes leading to gene mutation itself. This was not effectively shown at very high doses, where the longer-wave radiation may have saved the life of a disproportionally greater number of those cells into which an exceptionally large amount of uv had penetrated. At medium doses, however, the longer waves usually reduced the observed mutation rate to considerably less than half the value found with uv alone. — Similar results are reported by L. Altenburg (personal communication. — Work supported by grant from U.S. Public Health Service.

MICKEY, GEORGE H., Northwestern University, Evanston, Ill. The origin in a mosaic of a new third chromosomal dominant eye mutation in Drosophila melanogaster. — A new rough eye mutation arose in a single mosaic male among 102 progeny of a female treated with cold shock at an early embryonic stage. The mosaic male showed the expected dominant markers Pin and Blister-like but only the right eye was rough. When mated to a series of wild type females he produced 788 offspring, only one of which showed the rough eye trait. In numerous pair matings of sibs no rough eyed individuals appeared. The F_1 rough eyed male produced 50% offspring rough in both sexes. Crossing tests proved it to be a third chromosomal dominant gene. — Obviously the mutation occurred during cleavage in the mosaic fly, some of the mutant cell progeny going to produce the imaginal disc of the right eye and at least one mutant cell entering the testis thus

constituting a mosaic gonad. Various hypotheses have been tried to explain the very low proportion of mutant offspring of the mosaic male. The most plausible seems to be that at the time the gonad was formed, only a single mutant cell was included. Subsequently it failed to divide as rapidly as the others, or some of its progeny went to form the testis sheath, Sertoli cells, etc., so that perhaps only one or two mutant cells actually gave rise to germ cells.

MILLER, W.J., University of Wisconsin, Madison, Wis. The time of appearance of species specific antigens of Columba guinea in the embryos of backcross hybrids. — The species specific antigens A, B, C, E, and F of the red blood cells of Columba guinea, in contrast to C. livia, as present in backcross hybrids and in offspring of matings inter se of backcross hybrids, were demonstrable as early as blood cells could be obtained from the embryo - i.e. about the 29 somite stage or at 72 hours of incubation. All the specified antigens as found in the embryos and squabs were present in agglutinating strength approximately equal to that of control cells from mature birds, with the exception of antigens A and F which, although present in the embryonic cells, tended to disappear at the hatching of the squab. However, the red blood cells carrying A and F regained their titer and agglutination strength slowly during the first 2 weeks to two months after hatching. — It seems probable that these antigens of guinea first appear at the same time and stage of every individual and are maintained throughout life in approximately constant strength, with the exception of A and F. This view is supported by (1) realization of expected approximate proportions of embryos and squabs possessing the antigen as determined by the kind of mating involved, and (2) possession of the antigen by all offspring from birds homozygous for the antigen in mating with individuals lacking the antigen - regardless of the stage of development tested.

MILLER, W.J. and C.R. BRYAN, University of Wisconsin, Madison, Wis. Serological differentiation of the homozygote and heterozygote following a species cross in pigeons. — Following backcrosses to livia of the hybrids and selected backcross hybrids from the mating of Columba guinea and Columba livia, five antigens of the erythrocytes peculiar to guinea (called A, B, C, E, and F) have been obtained in unit form. These are produced by the action of one or more genes on separate chromosomes derived from guinea. Matings inter se among backcross birds carrying any specific antigenic substance, as C, have produced some individuals homozygous for that substance. The cells of the heterozygotes differed serologically from the cells of the homozygotes in that they absorbed all antibodies from anti-livia serum, whereas those of the homozygotes (CC) absorbed from this antiserum all antibodies except those for a substance in livia (called C′) presumably contrasted to the C substance of guinea. The C′ substance has been demonstrated in the cells of each of 68 pigeons tested

to date. Therefore, any anti-livia serum, or antiserum to the heterozygote, which contains antibodies to the C' substance is a source of a reagent in the differentiation of homozygotes (CC) from heterozygotes (CC'). — By parallel procedures, it has been possible to differentiate the homozygote from the heterozygote for each of the other specific characters of guinea, A, B, E, and F, and to recognize in livia and in the respective heterozygotes antigenic characters A', B', E', and F' contrasting to each of the specific guinea substances. — The genetic and serological tests for the recognition of the heterozygotes and homozygotes are in agreement.

MITTLER, SIDNEY, Illinois Institute of Technology, Chicago, Illinois. Variation in tumor formation in D. melanogaster reared on yeasts that do not require vitamins or amino acids. — Tu^{50j20} was found in inbreeding wild flies trapped in the Chicago area. 4.7% of the flies show an abdominal tumor if reared on the agar-cornmeal media innoculated with baker's or brewer's yeast Saccharomyces cerevisicae. Since tumor formation has been reported to be partly dependent upon the nutrition of the larvae and that the larvae must have yeast to grow, the larvae were reared on yeasts that require no amino acids or vitamins. A survey was made of yeasts that could live only on glucose, NH_4SO_4 and several trace elements. Tu^{50j20} was raised on the following yeasts in an amino acid, vitamin free media presented in a series decreasing in the ability to aid in the formation of tumors: Hansenula anomala, Pichia membranaefaciens, Candida sorbosa, Nadsonia fulvescens, Debaromyces globosus, Hansenula saturnus, Torulopsis utilis, Rhodotoryla gracilis, R. glutinis and Geotrichium. The flies reared on the amino acid and vitamin free media are believed to obtain practically all their nourishment from the yeasts and were fewer in number than those reared on the standard media. Also, the percentage of tumors was lower in flies reared on the vitamin and amino acid free media.

MULLER, H.J. and J.I. VALENCIA, Indiana University, Bloomington, Ind. The localization of the mutagenic action of neutron-induced ionizations in Drosophila. — The frequency of translocations induced by fast neutron irradiation of Drosophila spermatozoa was found to vary linearly with dose even at doses sufficient to produce multiple proton tracks per sperm. This shows that broken chromosome ends derived from different breaks caused by the same track undergo recombination with one another much oftener than with those of different tracks. Our interpretation is that breaks caused by the same track tend to occur near together, this proximity favoring union between the broken ends. Thus the pieces would usually unite before greatly changing their relative positions. It must further be inferred that in this material a break usually occurs close to the point of origin of the ionization that induces it, i.e., that remote breakage effects, resulting from migration of ionization-induced, relatively stable

mutagens over microscopically appreciable distances, are uncommon. — Further evidence for this conclusion is provided by the finding that loci, like that for white eyes, which with X-rays give "visible mutations" seldom accompanied by a lethal effect unless there is a microscopically visible deficiency or other rearrangement, give with neutrons "visible mutations" that are usually accompanied by a lethal effect, even when there is no cytologically demonstrable alteration. This concatention of two mutagenic effects in close proximity (whether in these cases usually breaks or gene mutations or both is not yet decided) would result from the crowding of ionizations in proton tracks, provided the mutagenic action of the ionizations remained narrowly localized. — Work supported by grant from American Cancer Society.

MUNDKUR, Balaji D., Southern Illinois University, Carbondale, Ill. The genetics of long-term adaptation of galactose by yeast. — Long-term adaptation to galactose is achieved by cells of Saccharomyces chevalieri after a period of six days contact with the sugar. Genetic analysis of hybrids involving S. chevalieri has confirmed the view that the adaptation, in this species, is determined by a gene g_S, allelic to G which determines a 24 hr., rapid fermentation of galactose. Adapted g_S organisms ferment the sugar as rapidly as do cells of G genotype, but can frequently be de-adapted by an intervening transfer to galactose-free substrate and reverted to the initial 6 day long-term adapting type. Exceptional clones are, however, encountered which fail to de-adapt in spite of extended periods of growth on galactose-free substrate. The nature of such clones was analysed. Adapted, rapid fermenter S. chevalieri haploids crossed with non-fermenter (g) haploids in glucose yielded ascus types:-

$$2\ G : 2\ g$$
$$2\ g_s : 2\ g$$
$$1\ G : 1\ g_s : 2\ g$$

Intra-ascus sibling crosses carried through three generations in glucose resulted in recurrence of these same tetrad classes, indicating that rapid fermenter segregant clones which fail to de-adapt and revert to long-term types comprise mixtures of G and g_S cells. These enter into mass copulations with g cells to yield the three tetrad types encountered. The transformation of g_s to G is mutant in nature. — In stocks of Saccharomyces haploids (SIU pedigrees) of composite origin, long-term adaptation is achieved by mutation of g cells to G. Outcrosses of such derived G cells to non-fermenters consistently yield Mendelian, 2 G : 2 g asci only.

NABOURS, R.K. and F.M. STEBBINS, Zoology Dept., Kansas Agricultural Expt. Station. X-ray induced translocations among the chromosomes of Apotettix eurycephalus Hancock. — Three x-ray induced translocations among the chromosomes of Apotettix eurycephalus have been manifested: 1. A portion of one of the number 1, smallest pair of autosomes bearing the closely linked genes for dominant color

patterns, O,T,G, was translocated to augment one of the middle sized, 4th autosomes. Both the deficient donor, number 1, and the accreted receptor, number 4 autosomes survived for experimental breeding and cytologic examination. 2. A portion of a number 1 autosome bearing the gene for the dominant color Pattern W, was transferred, this time to enlarge the X, sex chromosome. Only the accreted receptor survived for breeding and cytological investigation. (nabours, Stebbins and Robertson, 1933, 1941, 1950). 3. In the third translocation, previously unreported, the gene for a dominant pattern, T, also on the number 1, has been removed to another as yet unspecified autosome. Both the donor and receptor autosomes have survived for extensive breeding. Cytological examination is pending. This translocation has exhibited several aspects similar to 1, above, but also a few conspicuous differences. (a) No individual bearing the deficient donor, without the complemental accreted receptor autosome, has grown up for the record. (b) There have been a few instances of crossing over between the deficient donor, and thereby impotent autosome, and its homolog (bearing gene markers) which restored it in each instance to approximate normal potentiality. This did not occur in case 1, above. (c) Individuals bearing a homologous pair of deficient donor, and a pair of accreted receptor autosomes propagated large numbers. This also did not occur in case 1, above.

NEEL, J.V., I.C. WELLS, and H.A. ITANO, University of Michigan, Ann Arbor, Michigan, and the California Institute of Technology, Pasadena, California. Familial differences in the proportion of abnormal hemoglobin associated with heterozygosity for the sickle cell gene. — Previous studies have revealed the existence, primarily in Negro populations, of a gene which when heterozygous produces the asymptomatic sickle cell trait and when homozygous results in the clinically serious sickle cell anemia. It has also been shown that in individuals with the sickle cell trait, 25-45 per cent of the hemoglobin is of an electrophoretically abnormal type, whereas in patients with sickle cell anemia 90-100 per cent of the hemoglobin is of this same type. The structure of an important body protein is thus responsive to a simple genetic mechanism. In the present study, seven negro families in which the sickle cell trait is present have been investigated by the techniques of electrophoresis in an effort to determine whether there are significant differences between families as regards the proportion of abnormal hemoglobin associated with the trait. These seven families, especially selected because of their size and the availability of both parents for study, contain 74 individuals, of whom 32 are known to have the sickle cell trait and were studied. An analysis of the variation in the proportion of abnormal hemoglobin present into two portions, that between members of the same family, and that between family means, revealed a highly significant difference between family means. This difference between family means is thought to be genetic rather than environmental in origin, but the exact nature of the genetic control is not at present clear.

NEWCOMBE, H.B., Atomic Energy Project, National Research Council of Canada, Chalk River, Ontario. Sectoring in colonies from irradiated cells of Escherichia coli. — Colonies from ultraviolet- or gamma-irradiated cells of E. coli, grown on mannitol-tetrazolium agar, are frequently sectored for colour response. With ultraviolet doses of 500 to 1000 ergs per sq. mm. 15 to 20 per cent of the colonies were sectored, and with gamma doses of 40,000 to 80,000 r 6 to 7 per cent. This was at first thought to be due to the presence of more than one nucleus in the treated cells. However, if this were the correct interpretation one would expect to eliminate the sectoring by selecting the descendents from a single nucleus. To do this, selection for induced mutations to phage resistance was carried out on the mannitol-tetrazolium agar, the treated cells being allowed a short period of growth and then sprayed with a mixture of phages T1 and T5. Contrary to expectation, a proportion of the colonies arising after further incubation were still sectored for colour. This proportion, however, was roughly half that observed in the non-selection experiments. Essentially similar, although less extensive, results were obtained using streptomycin as the selective agent. — The sectoring which survives selection could be due to: (1) delayed mutation, (2) genetic interchange between two nuclei in the treated cell, (3) mutation in incompletely split chromosomes, or (4) mutation in hereditary particles other than the chromosomal genes.

NORMAN, A., University of California, Los Angeles, California. Nuclear interactions in ultraviolet irradiated Neurospora conidia. — The inactivation of Neurospora conidia by ultraviolet radiation is apparently a consequence of the inactivation of the nuclei. The inactivation cross section per nucleus is about twice as large in uninuclear (haploid) as in multinucleate conidia. The smaller cross section per nucleus in the latter is attributable to some sort of nuclear interaction. — Atwood (Genetics, 35, 95 (1950)) has shown that recessive lethal mutations contribute negligibly to the inactivation of the multinucleate conidia; the inactivation must be ascribed, therefore, to some non-genetic process. The frequency of recessive lethal mutation is high enough, however, to contribute significantly to the inactivation of the uninucleate conidia in which, of course, every lethal mutation is expressed. It is reasonable, thus to ascribe the difference in cross sections per nucleus exhibited by the uninucleate and multinucleate conidia to the expression of recessive lethal mutations in the former and not in the latter. On this hypothesis both genetic and non-genetic processes contribute to the inactivation of the uninucleate conidia. If the hypothesis is correct, then the genetic and non-genetic processes are strikingly similar. Both involve the nucleus, both processes exhibit the same action spectrum, the effects of both are reversed to about the same extent by photoreactivation, and, finally, the two processes are not independent; i.e., the production as well as the expression of recessive lethal mutations must be supressed by nuclear interaction in the

multinucleate conidia. (This work was done at Columbia University with the assistance of an A.E.C. Postdoctoral Fellowship.)

PAPAZIAN, H.P., University of Chicago, Chicago 37, Illinois. Multiple-gene control of incompatibility in a Hymenomycete. — Schizophyllum is a tetrapolar Hymenomycete, having two series of multiple factors controlling compatibility. About 30 years ago Kniep recorded that new incompatibility types, besides the four expected ones, were not uncommon among the spores of fruiting-bodies in this genus. He also noticed that the incompatibility type of vegetative monokaryotic mycelia was stable and that fruiting-bodies obtained by crossing "new factors" sometimes produced the ancestral factors, he attributed this to back mutation at the incompatibility locus. — The findings of Kniep have been confirmed and from the analysis of three exceptional tetrads as well as other data it is concluded that the A series of incompatibility factors is controlled by at least two loci such that crossing-over between them produces a new factor in the series. A different allele at a single locus is sufficient to produce a functionally different factor. — A low, but not too low, rate of crossing-over ensures the maintenance of a large number of different factors in a population without allowing too much inbreeding.

PIPKIN, SARAH B., School of Medicine, American University of Beirut, Beirut, Lebanon. Seasonal fluctuations in Drosophila populations at different altitudes in the Lebanon Mountains. — Seasonal fluctuations in Drosophila populations were observed at 6 collecting stations in the Lebanon Mountains. Owing to an October to May rainy season, D. subobscura, D. funebris, D. immigrans, D. hydei, D. buzzatii, D. busckii, and D. lebanonensis expanded their populations only once a year. With short life cycles, D. melanogaster and D. simulans expanded 2 and 3 times a year, respectively, at certain stations. Only Ain Anub in the upper maritime climatic zone supported all 9 common species in more than minimal numbers at some time during the year. D. buzzatii was a common summer species at the 3 maritime zone stations, being rare in the temperate altitude and continental zones. — Winter dormancy at the 4 higher stations is thought to be induced by (1) lessening of food supplies, (2) inability of flies to move about in search of food at temperatures of 50°F. or less, freezing minimum temperatures not being necessary. A given species reappeared following winter dormancy earlier in the spring at lower altitude stations and later at higher stations. The first spring appearance of a given species occurred at rather similar mean temperatures at the various stations. — D. simulans far exceeded D. melanogaster in numbers in 17 cases at a time of population expansion of one or both species, with mean temperatures ranging from 52°F. to 80.2°F. D. melanogaster far surpassed D. simulans in all 4 cases of a spring expansion following winter dormancy probably because D. melanogaster emerges earlier than its competitor from winter dormancy.

RAUT, CAROLINE, Detroit Institute of Cancer Research, Detroit, Michigan. Cytochrome deficient yeast strains. — In many strains of yeast apparently non-genetic, cytochrome deficient variants exhibiting a single strong alpha absorption band at 550 mu occur frequently ("petites"). A cross was made between one such strain and a genetically controlled cytochrome deficient strain (c) which is devoid of a cytochrome absorption spectrum except for a barely detectable band at 550 mu. In an ascus dissected from this cross, the following phenotypes for the four spore clones were observed: (1) wild type, (2) petite, (3) and (4) c. When a cross was made between petite and "c" spore clones within this ascus, only wild type and "c" progeny were recovered The petite character is not transmitted through a cross, although it is stable throughout many vetetative generations, as reported by Ephrussi et al (Ann. inst. Pasteur 76, 419-443, 1949). Furthermore, crossing by a cytochrome deficient recessive is capable of reconstituting the normal cytochrome phenotype in the petites.

RILEY, HERBERT PARKES, NORMAN H. GILES, JR. and ALVIN V. BEATTY, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. The oxygen effect on X-ray-induced chromatid aberrations in Tradescantia microspores. — Comparative X-ray dosage curves for the various types of chromatid aberratins (microspores examined at 24 hours following irradiation) have been obtained following exposures of inflorescences in atmospheres of pure oxygen and of pure helium. A constant X-ray intensity of 300 r per minute was used, and four dosages were administered — between 25 and 150 r in oxygen, and between 50 and 450 r in helium. A linear relationship between aberration frequency and dose was obtained for both isochromatid and chromatid aberrations in both the presence and the absence of oxygen. Aberration frequencies were higher in oxygen in both instances, but the magnitude of the difference was much greater for isochromatid types (2.6 x) than for chromatid types (1.4 x). Nonlinear relationships were obtained for chromatid interchanges in both the presence and the absence of oxygen, the exponents of the dosage curves being approximately the same (1.7) in both cases. For equivalent dosages, chromatid interchanges were almost four times as frequent in oxygen as in helium. Experiments have also been performed on the effect of intensity on aberration yield in the presence and in the absence of oxygen. In oxygen, a constant dose of 150 r was administered in 1/2 minute, 3 minutes, 15 minutes, and 30 minutes. In the absence of oxygen (in nitrogen), the same times of exposure were used, but a constant dose of 400 r was given in order to obtain comparable levels of biological response. There was no effect on intensity on the frequencies of chromatid and isochromatid aberrations. Chromatid exchanges decreased in frequency at lower intensities, generally similar types of curves being obtained for the two exposures. The results to date of the intensity experiments suggest that the average restitution time for broken ends is the same in the presence and in the absence of oxygen, and thus support the view that

oxygen exerts its effect by increasing the initial frequency of breaks rather than by modifying the behavior of broken ends during the recovery process.

ROTHENBUHLER, WALTER C., JOHN W. GOWEN, and O.W. PARK, Iowa State College, Ames, Iowa. Androgenesis in gynandromorphic hone bees (Apis mellifera L.). — Large numbers of bees having combinations of male and female tissue have been observed in the progeny of certain queens of a particular strain. Experimental matings have been used to test the various hypotheses advanced to explain these sex-mosaic individuals. Queens homozygous for the recessive ivory eye gene, when artificially inseminated with sperm from drones hemizygous for the independent recessive chartreuse eye gene, produce wild-type black-eyed females — both queens and workers. Drones from such matings are ivory eyed. Gynandromorphs, with no exceptions, had female facets black, and male facets chartreuse. This permits tentative acceptance of the hypothesis of the androgenetic origin of male parts from accessory sperm (bearing chartreuse) known to enter the bee's egg. Another queen homozygous for ivory eyes and inseminated with a mixture of black and ivory sperms produced both black and ivory workers in a definite ratio. On the basis of the androgenetic explanation of male parts and the usual zygogenetic explanation of female parts, sex of facets and color of facets in eyes of gynandromorphs would be expected to appear in all four possible combinations in a predictable frequency for each specific combination. Actual counts were in satisfactory agreement with expectation. Other matings add further confirmation.

RUBIN, B.A., Brookhaven National Laboratory, Upton, L.I., New York. The relation of nucleic acid metabolism to the genetic effect of radiation in bacteria.* — Levels of continuous radiation (x-rays or β-rays) which rapidly kill resting bacteria (E. coli B/r) permit the growth of metabolizing organisms. When resting cells are inoculated into a fresh medium the inception of growth (lag) is delayed as a direct function of the radiation dose rate; but when multiplication begins, the rate of increase of cell mass during continuous radiation is equal to that in unirradiated controls and the same final culture densities are reached. Average cell size increases with the number of generations that have occurred during irradiation, but this accounts for only a fraction of the cell mass, the logarithmic increase in viable count makes up the rest. — An examination of the mutant population (resistant to streptomycin) in cultures which have grown from 0 (resting) to 14 generations during irradiation indicates that the mutation rate is affected by the radiation dose, but not by the number of genetic reduplications during irradiation. Under the same conditions, changes in nucleic acid

*Research carried out at Brookhaven National Laboratory under the auspices of the Atomic Energy Commission.

metabolism appear which are related to the extent of synthesis that has occurred during the irradiation.

RUSSELL, W.L., J.C. KILE, JR. and LIANE B. RUSSELL, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Failure of hypoxia to protect against the radiation induction of dominant lethals in mice. — To test whether hypoxia would protect against radiation induction of dominant lethals in mice, groups of males were: (a) X-irradiated with 800r in air, (b) non-irradiated in air, (c) X-irradiated with 800r in a 5% oxygen, 95% helium mixture, or (d) nonirradiated in the same mixture. The gas was passed through the exposure chamber for five minutes prior to irradiation and during irradiation in exposed groups and for the quivalent time in non-irradiated groups. Females fertilized by these males shortly after treatment were opened just before term and their uterine contents examined. Classifying embryos as alive or dead (including resorption sites) gave the following mean numbers of the two respective types per litter, with from 47 to 50 litters in each group: (a) 3.9 and 3.6, (b) 8.2 and 0.98, (c) 3.6 and 3.5, (d) 8.3 and 0.96. Repetitions of the experiment with 30 minutes pretreatment in the same gas mixture and irradiation in this or in 2% oxygen 98% helium gave similar results. These data give no evidence for protection by hypoxia against the induction of dominant lethals to which the excess death of embryos from irradiated sires is attributable. This is in contrast to the protection against genetic damage found by others in various organisms. It is also in contrast to protection by hypoxia, in this experiment, against direct radiation effects on the exposed males: acute radiation death reduced from 92% to 0%, length of sterile period and graying of hair decreased.

SAWIN, P.B. and R.H. CURRAN, R.B. Jackson Memorial Laboratory, Bar Harbor, Maine. Racial variation in the pattern of reproduction of the rabbit. — Fertility, fecundity, milk production, maternal instincts, growth rate, and viability are the six major factors in efficient reproduction. Analysis of data gathered over the years 1948-50 on five races of rabbits inbred for 15-22 generations shows statistically significant differences in the pattern of reproduction indicating a genetic background. Race X has only mediocre fertility due to a large proportion of does which are hypogenital, a result of pituitary deficiency. It's fecundity, considering its body size, is good, and its milk production is normal and steady. With respect to its maternal instincts, it builds a precocious but poorer nest than average and otherwise appears normal. Individually it is highly agressive but as a race it has only the average interest in protecting its young. Its mortality tends to be at a maximum at a later age than in the other races. Race III is outstanding for its high fecundity, its early cessation of lactation (which may be a contributing factor to high juvenile mortality), and its nonagressive disposition; and Race V for its delayed lactation and below average interest in protection of young. Since most of these variations are known to be

primarily influenced by the pituitary and sex hormones and by their interactions, these races afford valuable genetic material for further technical studies of hormone interaction and their effects upon growth and the reproductive process and for practical studies directed toward, analysis of the factors in efficient reproduction, and toward breed or race improvement.

SCHWARTZ, DREW, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. A case of male sterility in maize involving gene-cytoplasm interaction. — The inheritance of a male sterile condition in maize was found to be conditioned by an interaction of three factors: (1) a dominant gene for male sterility (Ms_{21}), (2) a dominant suppressor of sterility (S^{Ga}) associated with a male gametophyte effect, and (3) a specific "sterile" cytoplasm ( ) essential for the expression of male sterility. Male sterile plants are of the constitution - Ms ms s^{ga} s^{ga}. Plants which either lack the dominant sterility gene, carry the dominant suppressor, or possess the nonsterile cytoplasm are male fertile. Due to complete selective fertilization, the suppressor gene segregates only when the heterozygote, S^{Ga} s^{ga}, is used as the pistillate parent. The inbred line Kys is homozygous recessive for ms and s^{ga}. All other unrelated lines studied were found to be Ms Ms S^{Ga} S^{Ga}.

SHACKELFORD, R.M., University of Wisconsin, Madison, Wisc. Color phases of the ranch-bred mink. — Fourteen mutant genes are known which alter the wildtype color of the ranch mink. Nine of these are recessive to their non-mutant allel: four produce a blue gray color, the aleutian (alal) being distinguishable from platinum (pp) and imperial platinum (ipip) which are phenotypically similar, but sometimes confused with steelblu ($p^{S}p$ or $p^{S}p^{S}$); three produce chocolate brown color phases called "brown-eyed" pastel (bb), "green-eyed" pastel (bgbg), and imperial pastel (bibi); the gene for albinism, c^{H}, allows for the development of a small amount of pigment, while the gene o produces a pattern termed "goofus" which bears considerable resemblance to the pattern of the Siamese cat. — Five mutant genes can express themselves in the heterozygous state. Thus substitutions of one of these in an otherwise wild type genome produces the pattern color phases commonly known as black cross (Ss), royal silver ($s^{R}s$ or $s^{R}s^{R}$), blufrost (Ff), ebony (Ebeb and colmira (Cmcm). Several color phases result from the combination of two or more of these mutant genes, the sapphire (alalipip or alalpp) and the "red-eyed" pastel (bb bgbg) being of most commercial importance at the present time.

SINGLETON; W.R., Brookhaven National Laboratory, Upton, L.I., New York. Effect of continuous gamma radiation on mutation rate in maize*. — Continuous gamma radiation from Co^{60} was used in

*Research carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

studying the mutations induced in maize pollen. The intensity varied from 125 r/day to .09 r/day. Four endosperm characters were studied R, Pr, Su and Sh. Pollen from the dominant stock A B Pl C R Pr Su Sh growing in the radiation field was placed on silks of a multiple recessive A b pl C r pr su sh growing in a field with only background radiation. — The following results were obtained. (1) There was no increase in mutation rate over the control when the radiation was less than 5 r/day. (2) Above 5 r/day there was a gradual increase up to 31 r/day with a more marked increase from 31 to 55 r/day. From 55 r/day to 125 r/day the increase in mutations was even more marked. The increase in mutation rate at the higher intensities of radiation was greater than expected if the increase is linearly proportional to dose. (3) The logarithm of the mutation rate seemed to show a linear relationship with dose received. (4) The gene with the greatest spontaneous mutation frequency (R) showed a smaller increase in induced changes than the other three genes Su, Sh and Pr. (5) In control plots the mutations detected in the microspore were appreciably higher than those found in the megaspore. This is expected since only those mutations that occur at or before the first mitotic division of the megaspore mother cell will be detected because of the triple fusion forming the endosperm.

SMITH, H.H., Cornell University, Ithaca, N.Y., Relation between sterility and morphological characters in an interspecific Nicotiana cross. — Interrelationships between morphological characters and pollen sterility were studied in the F_2 and first backcross generations of the interspecific cross Nicotiana Langsdorffii x N. Sanderae. The morphological characters used were three corolla dimensions that are determined by multiple factors. The combination of these characters in F_2 and backcrosses gives an estimate of the genetic composition of a phenotype with respect to relative amounts of parental germplasms. Pollen sterility is both chromosomal and genic; and many factors are involved in the inheritance as evidenced by the continuous variation in segregating generations. — F_2 segregation did not overlap the parental ranges in morphological characters and no correlation was observed between recombination types and pollen abortion. In the backcrosses some significant differences in pollen abortion among morphological recombination types were found and there was a tendency for segregants most like the recurrent parent to be least sterile. The results were interpreted to indicate that the genetic factors causing partial species isolation through gamete abortion are different from those governing the specific morphologies. Linkages occur between the two gene systems. — The data supported the interpretation that gamete elimination, as measured by pollen abortion, is relatively unimportant in differentially restricting morphological gene recombinations in F_1 gametes and F_2 segregants.

SMITH, W.K., University of Wisconsin and U.S. Dept. of Agriculture, Madison, Wisc. Chlorophyll-deficient interspecific hybrids in Melilotus.

— Hybrid seedlings from the cross between Melilotus alba and M. dentata, two species of the subgenus Eumelilotus, are almost devoid of chlorophyll and live only for a few days after emergence. The lack of chlorophyll in the F_1 hybrids constitutes an effective barrier between the species. Crosses have now been made between pairs of species among eight included in the Eumelilotus and reveal additional instances of this barrier within the genus. The eight species fall into three groups: Group A comprises the three species M. alba, M. polonica and M. suaveolens which give normal green hybrids when intercrossed; the four species in Group B, M. altissima, M. dentata, M. taurica and M. wolgica, give chlorophyll-deficient hybrids in crosses with M. alba. In contrast, pollinations between M. officinalis, the only representative of Group C, and any of the other seven species have not given hybrids.

STALKER, HARRISON D., Washington University, St. Louis, Mo. Diploid parthenogenesis in the cardini species group of Drosophila. — Unfertilized eggs of an undescribed Mexican species of Drosophila related to D. cardini may develop into normal, fertile diploid females. This occurs in about 1/1200 of the unfertilized eggs under standard laboratory conditions at 25°C. Under optimal temperature conditions approximately 1/40 of the unfertilized eggs begin development, and of these 9/10 die as unhatched or newly hatched larvae. There is little late larval or pupal mortality, so that, under optimal conditions about 1/400 of the eggs finally form adult females. As many as four consecutive generations of impaternate females have been produced. Virgin females heterozygous for a sex-linked mutant gene produce heterozygous and both types of homozygous daughters. It is suggested that the diploid chromosome constitution is reconstituted by fusion of second division products, or by an incomplete second meiotic division. The analysis is being continued. Four impaternate males have appeared, (about 1% of the impaternate progeny). A testis smear of one of these males shoed it to be XO; sterility of the other three suggests that they were XO also. — Two additional species in the cardini group: D. polymorpha and an undescribed species from Florida, both show some development of unfertilized eggs. In both of these species the phenomenon is much rarer than in the Mexican species mentioned above, and so far no impaternate individuals have survived the larval period, most dying prior to hatching.

STORMONT, C., University of California, Davis, Calif. An example of a recessive blood group in sheep. — Generally, the serologically reactive substances of erythrocytes are expressed in any genotype, although in the cells of heterozygotes there may be reduced amounts of one or both of a pair of antigens. Observations in this laboratory concerning the R-O blood-group-system of sheep are of interest in that they indicate the O substance is truly recessive. Previous studies by independent investigators have shown that two major blood groups called R and O comprise the R-O system of sheep. These groups have

been distinguished by means of normal antibodies (anti-R) found in certain sheep of group O. Hence, group O has been routinely characterized by the absence of reaction with anti-R. Further, it has been shown that R is inherited as a dominant in contrast with its absence, O. In this laboratory it has been observed that certain normal antibodies of cattle react selectively with the erythrocytes of sheep of group O. In tests of over 100 bloods with both anti-R and anti-O reagents, the R and O reactions have been mutually exclusive as would be expected if O is recessive to R. Further, it has not been possible to demonstrate O, by means of antibody-absorption, in the cells of sheep proven heterozygous for the two alleles. All immunogenetics tests to date agree with the conclusion that O of sheep erythrocytes is recessive to R. The only other known example of recessiveness in a blood-group character would appear to be the Lewis antigen of man.

STRONG, L.C., Yale University School of Medicine, New Haven, Conn. A cumulative change in susceptibility to induced fibrosarcoma in mice, associated with litter seriation. — In male mice of the inbred 2Prunt descent the median latent period for methylcholanthrene-induced fibrosarcomas is 171 days, with probable error ± 2.44 days, while in females it is 119 ± 1.30 days. Litter seriation analysis shows no significant trend in females (slope 0.76), but a progressive decrease in latent period with advancing litters for males. Males from first litters gave a median latent period of 223.7 days, while those from fifteenth litters gave 128.5 days. The trend in successive litters of males is a straight line with a slope of 5.8. Mice from advanced litters (seventh to tenth) have in turn produced offsprings resembling them in susceptibility. The sons of advanced litters had a median latent period of 148 ± 5.42 days, daughters 121.0 ± 3.21 days. Thus a non-Mendelian type of inheritance seems involved in transmission of susceptibility to fibrosarcoma.

STURTEVANT, FRANK M., JR., Northwestern University, Evanston, Ill. Studies on the mutagenicity of phenol in Drosophila melanogaster. — The following experimental treatments were carried out on the Oregon-R stock in an attempt to increase the spontaneous mutation rate by the administration of phenol: male third instar larvae and imagos of both sexes were subjected to phenol vapors for a twenty-four hour period; male third instar larvae were injected with 0.20%, 0.25%, and 0.50% solutions of phenol in Holtfreter's saline; female larvae were injected with 0.50% phenol solution; mature sperm were treated by administering a precopulatory douche of 0.01%, 0.10%, 1.00%, and 2.00% phenol solutions to virgin Muller-5 females. Each experimental series was examined for the presence of recessive lethal mutations by the Muller-5 method. In no series was the mutation rate different from that of the controls to a degree which was statistically significant. The reason for the failure of in vivo treatment is postulated to be phenol detoxication in the larva and inability of the phenol to reach the germ plasm during the critical physiological period.

SWANSON, C.P. and HENRY T. YOST, JR., The Johns Hopkins University, Baltimore, Md. The action of infrared radiation on the chromosomes of Tradescantia.* — Previous studies in Drosophila and Tradescantia have revealed that pretreatment with infrared significantly raises the frequency of x-ray induced aberrations; posttreatment acts similarly in Tradescantia but not in Drosophila. The hypothesis has been advanced on the basis of the Tradescantia studies that infrared, when used in combination with x-rays, raises the level of primary breaks, this in turn yielding a higher frequency of aberrations..... By analogy with certain physical phenomena such as phosphorescence it seems possible that infrared might act by raising the molecular structures of the chromosome to a metastable condition, in which state these structures, as yet unidentified, react with increased frequency to subsequent doses of x-rays. This was tested by subjecting infrared-treated buds to heat shock (48°C. for 30 sec.), a treatment designed to return a metastable state back to an original ground state. We have shown that such heat treatments, interposed between infrared and x-rays, remove the infrared effect, the chromosomes behaving to x-rays as though no infrared had been given. A similar effect was obtained when the order of treatment was x-rays - heat - infrared. From these two types of experiments, plus additional studies which revealed that the effectiveness of infrared was a function of the temperature of the buds at the time of infrared treatment, we have concluded that the primary action of infrared is to induce metastable states in the chromosomes. Such chromosomes react more readily with the free radicals released in the surrounding area by the ionizing radiation to yield a higher frequency of primary breaks per given dose of x-rays. The fact that the heat treatment can also prevent the action of infrared when infrared is given as a posttreatment to x-rays suggests that the x-rays induce, in addition to the usual visible alterations, a reservoir of loci which can be acted upon by the infrared and brought to the level of primary breaks, after which they can enter into recombination with other similarly broken ends. Whether these loci are to be considered incipient breaks, metastable states of a high reactive capacity, or some other category of change, is not clear at the present time. Heat shock, however, can eliminate them as a source of primary breaks, and subsequent doses of infrared can no longer affect them.

TAYLOR, J. HERBERT, University of Tennessee, Knoxville, Tennessee. Chromosome structure and behavior in relation to gene action. — In both fixed and living material, chromomeres are shown to be expressions of spiral structure, a bonding of certain adjacent gyres of a spiralled fiber. Comparisons of spiral structure during meiosis and mitosis in cultured anthers reveal that major spirals of meiotic metaphases and the standard spiral of mitosis are quite

*Supported by a grant-in-aid from the U.S. Public Health Service and a contract with the Atomic Energy Commission.

similar. Each is superimposed on minor spirals that differ in degree of tightness (compactness of gyres of less diameter). This is reflected in differences in major gyre diameter and number. Chromosomes are always spiralled to some extent and the minor spiral is present at all stages of meiosis. Diplotene begins with its tightening in a characteristic pattern along the chromosome. The resulting torsion produces relational coiling and the major spirals. The tightening of the minor spiral is postulated to result from rearrangements of a submino and a fourth set of spirals of the ultimate nucleoprotein fiber which would be about 200 times the length of an anaphase chromosome. The chromosomal cycle is characterized not by a continual unwinding of spirals, but by reversible changes in the tightness of spirals of variable character along the length of the fiber. These changes in spiralization, although influenced by the intranuclear environment, reflect structural differences along the length of the fiber which differentiate it into genetic loci capable of acting as templates for their own reproduction or for protein synthesis. In the tightly spiralled stages of division or in the chromomere condition of some resting stages these syntheses would be very limited or absent. Such a concept helps explain the inactivity of certain genes during stages of ontogeny, the position effect and unstable genes associated with heterochromatin, and makes other position effects, mutations, and the evolution of new genes as simple to explain as chromosomal aberrations.

VALENCIA, J.I. and J.T. MCQUATE, Indiana University, Bloomington, Ind. A cytogenetic analysis of 70 ultraviolet-induced X-chromosome lethals in Drosophila. — Seventy ultraviolet-induced lethals, obtained by McQuate from irradiations of mature sperm which produced more than 1.3% lethal mutations, were approximately localized by him by means of crossover tests, and the appropriate regions of the salivary chromosomes were then studied by Valencia. Three cytologically visible chromosome rearrangements were found: (1) an X-3 translocation that reduced crossover frequency and that had its lethal effect at or near the point of translocation but involved no detectable deletion, (2) a deletion of about 18 bands, and (3) a moderately small non-lethal inversion, with a separable lethal in the same chromosome. — Evidence of decidedly non-random distribution of the mutagenic effects among the treated individuals was found in the fact that the translocation and the deletion, as well as a third letha, were all found among the 47 tested daughters of a single male, which also produced a son having a deletion in its treated $y^3.Y^1$ chromosome, and that among the 29 tested daughters of the male which produced the inversion with its separate lethal, two other lethals were found. — These results, taken in connection with those previously reported by McQuate (these Proceedings, 1950), indicate that ultraviolet can induce, in Drosophila spermatozoa, breaks capable of recombination. However, a dose of X-rays of the same lethal-producing effectiveness would

probably have induced many more chromosome rearrangements. — Work supported in part by grants from American Cancer Society and U.S. Public Health Service.

VETUKHIV, M., Ukrainian Academy of Arts and Sciences, Inc., New York, N.Y. The factor of natural resistance to Tb in aminals. — The study of factors which cause the various degrees of natural resistance to tuberculosis in animals is a very important problem. One of the known factors is Ca-metabolism. — My research of the cattle showed: the breeds which originated in those districts where conditions encouraged good Ca-metabolism as a constitutional factor show better resistance to tb; (black soil districts, the ground water containing Ca, climate assisting better metabolism). — The young animals till the age of lactation or fecundation, when the Ca-metabolism changes, react to the infection in different ways. Under the same living conditions and the same possibilities of infection the young cattle showed 6% of positive reaction, but the grown-up animals gave 60%. — Studies of mice under the condition of experimental tb innoculation with the same doses and from the same stock of bacilli showed that death occurred much more often among the mice having poor Ca-metabolism than among the mice for which the Ca-metabolism was artifically heightened.

VICARI, E.M., R.B. Jackson Memorial Laboratory, Bar Harbor, Maine. Relation of epinephrine to the genic expression of fatal convulsive seizures in mice. — The optimum physiological threshold, for the onset of fatal convulsive seizures, induced by sound of 80-90 decibels, was found to be at the age of 25-34 days for the DBA inbred strain of Mus musculus. In an attempt to locate the underlying cause of the onset of convulsive seizures a series of compounds were used to protect the animal from fatal seizures at the most susceptible age. From a group of ten compounds epinephrine was found most affective in its protective prevention of fatal seizures, not only did it prevent death after seizures but also prevented the onset of convulsive seizures. — Around 350 animals of ages 28-34 days were used in this experiment. The smaller doses produced a 90 per cent protection up to 12 hours after treatment and after this the protection gradually decreased. The larger doses however were still highly protective at 24 hours.

WALSH, M.P., Boston College, Chestnut Hill, Mass. Chromosome variations in Lumbricus terrestris. — All the various criteria that have been used to establish the existence of polyploidy in cells were used in this study. No multiplication of chromosome sets beyond the diploid number was observed in the sex cells of the testes and seminal vesicles. Nothing like the polyvalent spermatogonia, spermatocytes and giant spermatozoa that have been found in a few other closely related species were found in this species. The variations in the size of

nuclei and nucleoli that were seen might be due to the physiological conditions of the particular cells in question and not to polyploidy. This study would seem to substantiate White's statement that polyploidy plays a very small part in the evolution of chromosomes of hermaphrodites. — As in other hermaphroditic groups like the cestodes and trematodes, variations due to aneuploidy and especially to the addition of single chromosomes were found in Lumbricus terrestris. Eighteen was the haploid number generally observed in the first meiotic metaphase plates and 36 was the diploid number in the spermatogonial divisoins. There were, however, a number of first meiotic plates that showed 17 or 19 tetrads and a number of spermatogonial divisions that showed variations from 34 to 38 chromosomes. The haploid number 16 has been established by other investigators for species of oligochaetes closely related to Lumbricus terrestris. — Non-pairing of homologous chromosomes which was witnessed frequently may account for the unequal distribution of chromosomes in anaphase so that one daughter cell receives both univalents. Although it is difficult to believe that polyploidy is important in the evolution of chromosome compliments in the oligochaetes, further investigations might indicate that polysomy has played a great part in the speciation of oligochaetes as it has in some other hermaphrodites.

WEIR, J.A., University of Kansas, Lawrence, Kansas. Ruby-spotted, a new recessive mutation in the house mouse. — The mutation first appeared as 3 piebald-type white spotted mice in a litter of 10 in the non-inbred MacArthur strain. The parents, which were 18% related, were ccDdBba^{t}a?♂ and CCDdbba^{t}a?♀ . The gene has proven to be recessive with belly spots sometimes evident in heterozygotes. Both eyes are ruby in all homozygotes but there is no diluting effect on black or chocolate coat colors. Incisors erupt normally but mice fail in condition at about 20 days and die at weaning. Attempts are in progress to determine the nature of the pleiotropic syndrome and to prolong life of affected individuals. The mutation may have occurred before the strain was obtained 7 generations previously. Pygmy and an obese condition have also appeared in the MacArthur strain.

WHITE, M.J.D., University of Texas, Austin, Texas. Supernumerary chromosomes in the Trimerotropine grasshoppers. — Supernumerary chromosomes have been found in ten species of Trimerotropine grasshoppers. In each species their frequency varies from one population to another, and may be zero in certain geographic areas. Three cytological types may be distinguished: acrocentric supernumeraries, isochromosomes and a type which is interpreted as an isochromosome with a large interstitial deletion in one arm (the last type has only been found in Trimerotropis inconspicua). In Trimerotropis sparsa, where both acrocentric supernumeraries and isochromosomes may be present in the same individual, no pairing between them takes place, although two acrocentries will regularly pair

to form a bivalent and two isochromosomes may do so (more frequently, the isochromosomes form univalents with a single chiasma between their arms). The lack of pairing between the two types of supernumeraries suggests that an acrocentric supernumerary is not simply equivalent to one arm of an isochromosome. All three types of supernumerary are largely heterochromatic, but contain interstitial euchromatic segments. Chiasma formation is apparently restricted to these euchromatic segments. The role of the supernumeraries in most populations is interpreted as an adaptive one, small numbers of supernumeraries being probably beneficial to the individual carrying them, while larger numbers are presumably deleterious. Such an interpretation is difficult to accept, however, in the case of a population of Trimerotropis gracillis, where the supernumeraries occur in less than 0.5% of the population.

WITKIN, EVELYN M., Carnegie Institution, Cold Spring Harbor, N.Y. Nuclear segregation and delayed expression of induced mutations in Escherichia coli. — Populations of strains B and B/r of E. coli were found to have different ratios of uninucleate to multinucleate cells at different stages of the culture cycle. A correlation was found between these ratios and the ratios of intact to sectored lactose-negative mutant colonies obtained on eosin-methyleneblue medium after ultraviolet irradiation. This was interpreted as evidence that nuclear segregation from heterocaryotic cells is primarily responsible for sectoring. It was shown that sectors can arise also by mechanism other than nuclear segregation, in experiments in which irradiated populations were plated on EMB, permitted to undergo several divisions, and then sprayed with bacteriophage T1. Phage-resistant mutants obtained on these plates are presumably derived from single nuclei, and should exhibit no lactose-negative sectors if the latter arise only by nuclear segregation. A small number of sectors was found in these experiments, as well as in parallel experiments in which an irradiated population of a streptomycin-requiring strain was plated on EMB, and screened for lactose-negative, streptomycin-independent mutants. Sectors obtained in the double-screening experiments could be due to: 1) segregation of extranuclear particulate units, 2) delayed mutation, or 3) genetic exchange between nuclei, chromosomes or strands. Analysis of these results indicates that phage-resistant and streptomycin-independent mutants also occur as sectors, and that physiological lag cannot entirely account for delayed expression of these mutations. The frequency of double mutants was found to be greater than would be expected by chance.

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